Volume 27, Number 5, April, 1955

The Yale Journal of Biology & Medicine

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OF BIOLOGY AND MEDICINE

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Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $6.00 in the United States, $7.50 in other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

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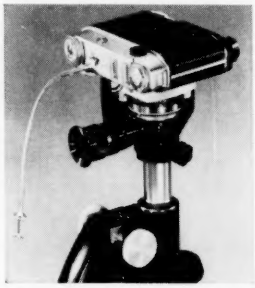

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PULMONARY RESECTION IN THE THERAPY OF TUBERCULOSIS. SOME LATE FOLLOW-UP STUDIES

INTRODUCTION

In his classical dissertation concerning artificial pneumothorax published in 1882, Forlanini[8] considered the possibility of resection for pulmonary tuberculosis and concluded that it was a desirable procedure on theoretical grounds. He predicted its future usefulness when the technical deficiencies of contemporary surgery were overcome. Within the past two decades those technical problems largely have been solved, and the resectional methods are being applied to an increasing number of patients with pulmonary tuberculosis. Not a few clinics have abandoned the collapse procedures entirely in their initial enthusiasm for the more direct approach of resection.

Pulmonary tuberculosis is all too often a disease characterized by late exacerbation, and one must ask if the modern program of long-term chemotherapy and resection has proved itself entirely adequate. Sufficient time has now passed so that adequately long-term evaluation of the results in the earliest cases can be made.

HISTORICAL SUMMARY

Ruggi[11] in 1883 and Tuffier[14] in 1891 were apparently the first to perform local pulmonary resections for the apical manifestations of tuberculosis. The results were inevitably poor. Gradual improvements in anesthesia techniques, the increasing availability of preserved blood, a growth in knowledge of detailed bronchovascular anatomy and pulmonary physiology, an increasing and favorable experience with resections for bronchiectasis and neoplasms, and finally the introduction of the potent antibiotics have all contributed to the development of resectional therapy in tuberculosis cases.

In this country, the first planned resections for tuberculosis were reported by Freedlander[5] in 1935 and by Dolley and Jones[6] in 1939. A review of the pertinent literature by Thornton and Adams[13] in 1942 dis-

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Received for publication February 21, 1955.

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closed a mortality rate of 44.8 per cent in 29 cases of pneumonectomy and 25.5 per cent in 46 cases of lobectomy. These results were discouraging, but Churchill and Klopstock[3] pointed out the following year that tuberculosis had not been diagnosed preoperatively in 30 of these collected cases and that the technique of mass hilar ligation had been employed in most. Satisfactory results could hardly have been expected. Churchill and his coworker reported an additional six cases performed by the preferable anatomic dissection method with survival of all. Overholt[9] in 1946, Sweet[12] in 1946 and 1950, and Bailey[1] in 1949 likewise reported favorable early results.

The technique of segmental resection, as introduced by Churchill and Belsey[4] for the bronchiectatic lingula, has been extended and applied to the tuberculosis problem by Chamberlain[2] and Overholt[10] among others. Long-term follow-ups were not emphasized in these earlier contributions, with the exception of Sweet's second paper, for the obvious reason that insufficient time had elapsed since the operations.

CRITIQUE AND INDICATIONS FOR RESECTION

Resection in some conservative form appears to be a more logical and preferable procedure than surgical collapse measures. The ablation of the major areas of disease should prevent or minimize the further spread of disease by bronchial, lymphatic, and vascular pathways. Resection may be more conservative of pulmonary function than thoracoplasty. This is especially true of upper lobe segmental resections for apical-posterior disease, of lower lobectomy as opposed to lower stages of thoracoplasty, and of cases where the extent of disease demands bilateral surgical procedures.

Resection is cosmetically preferable to thoracoplasty and less disturbing to shoulder girdle function. Resection can considerably shorten a patient's hospitalization period and total convalescence. One must not forget, however, that resection is only one phase in the treatment program. Proper sanatorium-type care with bed rest both before and after the surgical procedure cannot yet be abandoned.

Accumulating experience in the past decade has amply demonstrated that certain types of disease which are notoriously refractory to surgical collapse measures can be handled more efficaciously by resectional procedures. The following are generally accepted indications for resection:

1. Failure of thoracoplasty to close cavities or convert the sputum to negative.
2. Tuberculosis complicated by bronchial stenosis and by bronchiectasis.
3. Cavitary and fibrocaseous disease persisting after prolonged and adequate antibiotic treatment, with or without positive sputum.

4. Totally destroyed lung, especially when associated with bronchopleural fistula and empyema.
5. "Tuberculoma," the discrete round focus.
6. Tuberculosis associated with tumor or cases in which the differential diagnosis cannot be established without surgical exploration.

Adequately lengthy preparation with streptomycin, para-amino-salicylic acid, and isoniazid has permitted the indications for resectional therapy to be broadened and to include some formerly rejected cases. The drugs also have diminished the hazards of operations.

MATERIAL AND METHODS

The 130 patients who are the subjects of this report were admitted to the thoracic surgical section of the Grace-New Haven Community Hospital between March, 1934,

TABLE 1. RESECTION FOR PULMONARY TUBERCULOSIS

Male, 62	White, 124
Female, 68	Negro, 6
Age: Oldest, 74 years	
Youngest, 16 years	
Symptomatic duration: Briefest, 1 month	
Longest, 35 years	
Endobronchial disease, 37 = 27.4%	
Contralateral disease, 81 = 62.3%	
Sputum positive before operation, 78 = 60%	
Operative risk: Good, 76; Fair, 48; Poor, 11	

and June, 1953. Sixty-two patients were male and 68 female. There were 124 white and six Negro patients. The ages at time of surgery ranged between 16 and 74 years. Eighty-eight, or 67.6 per cent, were less than forty years of age.

The recognized length of illness before surgical intervention varied from one month to 35 years. Evidences of endobronchial tuberculosis were demonstrated at some time prior to resection in 37 cases or 27.4 per cent. Contralateral disease of some degree was noted in 81 or 62.3 per cent. Positive sputum within a month prior to resection was recorded in 78 or 60 per cent of the total. The estimated operative risk took into consideration the patient's age, his general condition, the existence of complications, and the functional ventilatory capacity. The risk was categorized as good in 76, fair in 48, and poor in 11 cases prior to resection (Table 1).

One hundred and thirty-five resections were carried out in these 130 patients, five cases undergoing a second procedure. Local (wedge) resections alone were performed in four instances, segmental resections in 26, lobectomy in 54, bilobectomy in four, and pneumonectomy in 47.

The total series has been divided for comparison and analysis into two groups: the first, a series of 40 patients operated upon before February, 1950, and already reported in the literature[7]; and the second, 90 cases who were subjected to surgery between February, 1950, and June, 1953.

The first seven cases were operated upon before streptomycin became available; and only 11 of the initial group received the drug preoperatively, the length of this preliminary course being very brief. In contrast, the patients in the second group of 90, with a few exceptions, received prior to surgery a minimum of six to nine months

TABLE 2. INDICATIONS FOR RESECTION

	Type of operation					
Indication	*Local excision*	*Segmental*	*Lobectomy*	*Bilobectomy*	*Pneumonectomy*	*Total*
Fibrocaseous residuals	0	24	32	0	6	62
Thoracoplasty failure	0	0	4	1	20	25
Bronchiectasis-Bronchostenosis	0	0	7	2	11	20
Uncontrolled cavity	0	0	4	1	5	10
Tuberculoma	4	2	4	0	0	10
Tumor suspect	0	0	3	0	3	6
Destroyed lung-empyema	0	0	0	0	1	1
Others	0	0	0	0	1	1
Totals	4	26	54	4	47	135

of sanatorium care with semi-weekly doses of one gram streptomycin and daily para-amino salicylic acid. During the hospitalization for surgery, a period which averaged about 15 days, streptomycin was administered daily (one gram) with PAS (8 to 12 grams). After return to the sanatorium, the semi-weekly streptomycin regimen was resumed for an additional three months to a year or longer depending upon the extent of preoperative disease and the clinical, radiological, and bacteriological responses to surgery.

The first two patients in the early group were operated upon employing the torniquet mass-ligation method. All other cases, with the exception of four wedge resections, were subjected to a meticulous anatomic dissection of the broncho-vascular structures. Twenty-five patients came to resection after a previous thoracoplasty had failed to control their disease, and 35 others required a limited "cap" or more extensive thoracoplasty as a space filling procedure after lobectomy or pneumonectomy. Five phrenic

nerve operations were carried out to limit overdistension of remaining lung. Sixty-five patients had no type of collapse procedure as a part of their surgical program.

In Table 2 are listed the indications for resection in the 135 operations and the types of surgery employed. Sixty-two resections were performed for residual fibrocaseous disease. Significant disease beyond segmental confines or fibrosis of a degree to preclude function made segmental procedures impossible or impractical in 38 cases of this group. Twenty-five patients represented thoracoplasty failures, and bronchiectasis with or without bronchial stenosis was the indication in twenty. Uncontrolled cavity, sometimes with tension, was the reason for surgery in ten; and tuberculoma was the diagnosis in another ten. Six patients were suspected of harboring tumor, but pathological study of the resected specimens revealed tuberculosis in some form. One was a tuberculoma, one was a lung abscess with tuberculosis, and four were fibrocaseous infiltrations. Destroyed lung with bronchopleural fistula and empyema was the indication for resection in one instance, and another case was misdiagnosed as bronchiectasis in 1936.

COMPLICATIONS OF SURGERY

The occurrence, more particularly in the early days of resection, of certain complications after surgical excision of tuberculous lung tissue, has been a factor of some significance in tempering unbridled enthusiasm for the method. The major complications include bronchopleural fistula, tuberculous or mixed empyema, and parenchymal spread of the disease. Bronchopleural fistula occurs with greater frequency after resections for tuberculosis than after resections for non-tuberculous diseases such as bronchiectasis and tumor. This tendency probably is related to existing disease in the bronchial wall or in the mediastinum at or near the site of bronchial transection. The incidence of this complication has been sharply reduced because of more prolonged and intensive antibiotic therapy before surgery. Fistula is likely to be associated with empyema and unexpanded lung. In fact, postoperative empyema is exceedingly rare in the absence of a proved fistula. Parenchymal spread of tuberculosis may be associated with fistula and empyema, but also follows on imperfect anesthesia technique which allows bronchial dissemination of infected sputum. Occasionally a so-called spread is caused by postoperative reactivation of diseased areas erroneously considered to be quiescent.

A comparison of data from the older group of 40 and the more recent group of 90 cases shows the expected steady decline in the three major complications (Table 3). The incidence of fistula has declined from 12.5 to 5.6 per cent, and that of empyema from 15.0 to 3.3 per cent. Postoperative spread occurred in four, or 10 per cent, of the first 40 cases but in only 4.4 per cent of the last 90. Wound infection of a minor or major degree was a finding in 12.5 per cent of the first and only 4.4 per cent of the second

group. Transient air leaks without clinical pleural infection occurred after six segmental resections in the past three years. Fortunately, mediastinitis and pericarditis, which were very rare complications in the first group, have not occurred in the second group.

The total number of cases suffering postoperative recognized complications in the first group of 40 cases was 12, or 30 per cent. In the second group of 90 this figure, including the cases of transient air leak, was 20, or 22.2 per cent. For the total 130 cases (representing 135 operations) 24.6 per cent suffered complications of major or minor nature.

TABLE 3. COMPLICATIONS OF RESECTION—TWO GROUPS

	First group 40 cases		*Second group 90 cases*		*Total 130 cases*	
Type of complication	*No.*	*%*	*No.*	*%*	*No.*	*%*
Bronchopleural fistula	5	12.5	5	5.6	10	7.7
Empyema	6	15.0	3	3.3	9	6.9
Spread	4	10.0	4	4.4	8	6.1
Wound infection	5	12.5	4	4.4	9	6.9
Transient air leak	.	...	6	6.7	6	4.6
Mediastinitis	2	5.0	0	0.0	2	1.5
Pericarditis	1	2.5	0	0.0	1	0.8
Total complicated cases	12	30.0	20	22.2	32	24.6

The performance of a resection of a greater magnitude than that contemplated as a result of the preoperative studies is not usually considered as a complication, but certain of these should be. An increase in the extent of proposed surgery may be required for technical reasons, or because of the discovery at exploration of more extensive areas of disease than were evident on clinical and particularly radiological investigation. On eight occasions in this series of 135 thoracotomies, the extent of the disease uncovered at the time of surgery indicated a larger resection than originally had been contemplated. In five other cases technical factors were the reasons. Three of the latter cases had such extensive induration and peribronchial infection at the segmental level that the procedure was advanced electively to lobectomy; vascular tears with hemorrhage necessitated the more extensive resection in the other two. An additional patient, a cancer suspect, had a pneumonectomy performed because of a misdiagnosis by the pathologist of the frozen section biopsy; the final diagnosis, however, was tuberculosis. In summary, 14 or 10.5 per cent of the operations were associ-

ated with more extensive resections than originally intended; five of these, or 3.7 per cent of the total operations, were related to technical factors.

RESULTS OF RESECTION

The early and late results of resectional therapy in the 135 operations carried out upon 130 patients in this series are summarized in Table 4 and Table 5. A report[7] was made on the first 40 cases in June, 1950. There were no hospital operative or postoperative deaths in this first group, but

TABLE 4. STATUS OF FIRST 40 CASES—(DEC. 1953)

Duration of follow-up	*Living*				*Dead*			
	Well	*Convalescent*	*Reactivated*	*Total*	*Post-operative*	*Late T.B.*	*Late Other*	*Total*
10 years	1	0	0	1	.	0	0	0
7–8 years	1	0	0	1	.	0	0	0
6–7 years	1	0	0	1	.	0	0	0
5–6 years	9	0	2	11	.	0	2	2
4–5 years	10	0	0	10	.	0	0	0
3–4 years	10	0	0	10	.	0	0	0
2–3 years	..	.	.	..	.	0	0	0
1–2 years	..	.	.	..	.	3	0	3
6–12 mos.	..	.	.	..	.	1	0	1
0– 6 mos.	..	.	.	..	.	0	0	0
Totals	32	0	2	34	0	4	2	6

subsequent to discharge five had died, four of tuberculosis and one of heart disease. Thirty-five patients remained as subjects for long-term follow-up. Since 1950 one additional patient has succumbed to unrelated and non-tuberculous causes. Thirty-two or 94.1 per cent of the 34 survivors (80 per cent of the original 40) represent arrested cases with negative sputum. These patients are working or carrying out domestic duties at home. The other two cases, both reported as arrested in 1950, have since suffered reactivation of the disease with positive sputa. In both, the reactivation was diagnosed in the sixth year after the resection, and they are undergoing further treatment.

The second and later group of 90 cases is too recent to afford long-term evaluation. The longest follow-up period (as of December, 1953) is 42 months and the shortest, five months. Eighty-five of the 90 were living at that time and five dead. Three patients succumbed to operative or post-

operative complications, and two died subsequent to discharge of unrelated causes. The operative mortality of the second group was therefore 3.3 per cent. Sixty-four patients, or 75.3 per cent of the 85 survivors, were considered to be arrested by clinical, radiological, and bacteriological criteria; they were either working or carrying on light housework. Twenty-one other cases, all in good general condition, were still convalescing, 16 at home on graded exercise programs and five in the sanatoria.

Considering the entire series of 130, 119, or 91.5 per cent, are living and 11, or 8.5 per cent, are dead. Three, or 2.3 per cent, were operative or post-operative hospital deaths, and eight were late deaths after hospital dis-

TABLE 5. STATUS OF LAST 90 CASES—(DEC. 1953)

Duration of follow-up	*Living*				*Dead*			
	Well	*Convalescent*	*Reactivated*	*Total*	*Post-operative*	*Late T.B.*	*Other*	*Total*
3–4 years	3	0	0	3	.	0	0	0
2–3 years	13	1	0	14	.	0	0	0
1–2 years	38	8	0	46	.	0	0	0
6–12 mos.	10	5	0	15	.	0	2	2
0– 6 mos.	0	7	0	7	3	0	0	3
Totals	64	21	0	85	3	0	2	5

charge. Four of the late deaths were caused by the tuberculous process, and four were entirely unrelated. Ninety-six patients, or 73.8 per cent, are arrested by radiological and bacteriological criteria.

As previously stated, only two cases of the original forty resected cases have suffered late reactivations of the disease. This represents about six per cent of the 34 survivors in that early group after a follow-up interval of four years or more. The present data on the second group of 90 cases lead to the belief that the late results in this group will be at least as satisfactory as the first, particularly since the antibiotic program in these later cases has been more adequate. It is well to remember, however, as the two long-term relapsing cases so well illustrate, that tuberculosis is a chronic disease with no respect for arbitrary time limits. Definitive follow-up data should be presented in terms of a decade or more.

SUMMARY

1. A series of 130 consecutive cases of pulmonary tuberculosis treated by resectional methods is reviewed.

2. The first 40 cases completed prior to February, 1950, and the last 90 cases completed prior to June, 1953, have been analyzed as groups for purposes of comparison.

3. The indications for resection are discussed.

4. The operative mortality of the 135 procedures was 2.2 per cent; the case mortality, 2.3. There were eight late deaths after discharge from the hospital; four of these were attributable to tuberculosis.

5. Thirty-two of the first forty cases, or 94.1 per cent of the 34 survivors in this early group, are considered to be arrested and well.

6. A comparison of postoperative complications in the early and late groups shows a very significant reduction in most categories.

ACKNOWLEDGMENT

The authors again wish to express their appreciation to staff members at Laurel Heights, Uncas-on-Thames, and the Gaylord Farm Sanatoria, and to the several private physicians who have co-operated in the care and follow-up examinations of their patients.

REFERENCES

1 Bailey, C. P., Glover, R. P., and O'Neill, T. J. E.: Comparison of results in two hundred consecutive resections for pulmonary tuberculosis. J. Thorac. Surg., 1949, *18*, 36.

2 Chamberlain, J. M. and Ryan, T. C.: Segmental resection in pulmonary diseases. J. Thorac. Surg., 1950, *19*, 199.

3 Churchill, E. D. and Klopstock, R.: Lobectomy for pulmonary tuberculosis. Ann. Surg., 1943, *117*, 641.

4 Churchill, E. D. and Belsey, R.: Segmental pneumonectomy in bronchiectasis; the lingula segment of the left upper lobe. Ann. Surg., 1939, *109*, 481.

5 Freedlander, S. O.: Lobectomy in pulmonary tuberculosis. J. Thorac. Surg., 1935, *5*, 132.

6 Jones, J. C. and Dolley, F. S.: Lobectomy and pneumonectomy in pulmonary tuberculosis. J. Thorac. Surg., 1939, *8*, 351.

7 Lindskog, G. E. and Law, F. D.: Pulmonary resection for tuberculosis. A report of 40 consecutive cases. Yale J. Biol., 1951, *23*, 474.

8 Lojacono, S.: Translation of Forlanini's original communication on artificial pneumothorax. Tubercle, 1934, *16*, 61.

9 Overholt, R. H., Langer, L., Szypulski, J. T. and Wilson, N. J.: Pulmonary resection in the treatment of tuberculosis: present day technique and results. J. Thorac. Surg., 1946, *15*, 384.

10 Overholt, R. H., Woods, F. M., and Ramsay, B. H.: Segmental resection; details of technic and results. J. Thorac. Surg., 1950, *19*, 207.

11 Ruggi, G.: *La tecnica della pneumectomia nell'uomo.* Bologna, 1885.

12 Sweet, R. H.: Lobectomy and pneumonectomy in the treatment of pulmonary tuberculosis. J. Thorac. Surg., 1946, *15*, 373; 1950, *19*, 298.

13 Thornton, T. F. and Adams, W. E.: The resection of lung tissue for pulmonary tuberculosis. Internat. Abstr. Surg., 1942, *75*, 312.

14 Tuffier, T.: *Chirurgie du poumon, en particulier dans les cavernes tuberculeuses et la gangrène pulmonaire.* Paris, Masson et Cie., 1897, p. 31.

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PROPRIETARY MEDICINE STAMPS DURING THE CIVIL WAR PERIOD†

In recent years physicians have tended to sit back in a complacent manner somewhat falsely confident that the pure food and drug act had taken care of or will take care of any fake or hoax perpetrated on the people. One has only to acquire a television set, however, to have an intensive course in pharmacology—to become aware of BC and AC recommended by doctors everywhere, of K_2 which destroys harmful bacteria, and many other of the newer medications. One evening of television is an indoctrination in medicine so graphic that it is small wonder that our patients are confused. We have made progress and of that there can be no doubt. We still have the proprietary product and the inferences that it is a panacea for all our ailments. But although it may seem that people reach out more frequently for panaceas in these times, I must remind you of some of the patent medicines in use a long time ago. I became interested in them through the tax revenue stamps affixed to them.

Stamp collecting is one of those sedentary avocational interests that people employ to "occupy one's mind." It can also be employed to make a person feel he has achieved some individual difference from others, yet not so much as to mark him as peculiar. Philatelist is the scientific term used to designate a stamp collector and literally translated this means "lover of labels." It can readily be seen why a psychiatrist would become a stamp collector for there is no branch of medicine that can compare with psychiatry in the use of neologisms and polysyllabic words.

One of the troubles with stamp collecting is that so many people collect and one has therefore to specialize in some unusual area in order to maintain an individual difference. This is one of the reasons why twenty-five years ago I became interested in collecting tax revenue stamps on medicine, for very few collectors give them much attention. Since the stamps had medical interest, it seemed easy for me to rationalize the idea of collecting them, although a psychiatrist should have no need of rationalizing anything in himself since he is presumably so well adjusted!

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† Presented at the Beaumont Medical Club, October 29, 1954.

Man has always been interested in health. He uses an expression of the state of his health to meet almost any situation with which he is faced, boasting about his health, for example, at the very moment he is apprehensive. One has only to watch the number of people frequenting the drug counters to have it confirmed that this is man's chief interest. If you try to reach the counter in such a store as Macy's in New York, you will discover that you indeed need the vitamin capsules you perhaps purchased to replace the energy you expended in procuring them. It is a conservative estimate that somewhere between seventy-five and a hundred million dollars are expended annually for aspirin, nose drops, and cough syrups. From the autumn of 1949, when antihistamines first were put on the market, through 1950 something like twenty-five millions were spent on these products.

Man's doubt of himself, his fundamental insecurity, probably is the chief factor in keeping him so closely tuned to his body mechanisms and his physical state. He does not want to be ill nor considered to be weak. Yet if there is a weakness, it can help to explain failure. If he is "Under prescription" or must take some medication, he has even more obvious evidence that can be mentioned in case he does run into a situation in which he does not conduct himself vaingloriously. During the war when I was in training stations, I found that hundreds of men coming into service brought with them their own medicine chests. This was not limited merely to a bottle of cathartic pills forced on a young adult by an oversolicitous mother. Rather, there were pills, tonics, liquids, and salves guaranteed to ward off anything from convulsions to snake bite.

Man is susceptible to the use of the drug—whether it be a love potion to pour into his sweetheart's Coca-Cola or something to build him into another Tarzan who can make strange noises as he swings on tree branches, it makes no difference. He likes to take things. The people who made drugs in 1860 were aware of this weakness. The best way to sell any product has always been to give it a health slant. The manufacturers of drugs of this earlier day were well aware of the psychological appeal of health.

As a result of a need for additional revenue brought about by the Civil War, Congress passed a revenue act in 1862 putting a tax on matches, proprietary medicines, perfumes, and playing cards. This, in itself, is not unique. Congress is always able to find something to tax. On this occasion though, they allowed the companies to print their own stamps at, of course, their own expense. Many companies were very willing to do this because they saw the advertising value of using their own slogans on the stamp. Also, they got a discount of from five to ten per cent on the orders from the dies that were printed. These stamps are now known as private propri-

etary revenue stamps. Two hundred and seventy-seven companies put out their own stamps and they are found in four different issues, old paper, silk paper, watermarked, and pink paper.

The tax on medicine was one cent for each twenty-five cents of the retail price of the product up to one dollar and two cents for each fifty cents or fraction thereof above that amount. This is of interest when you think of the lower prices of years ago; and when you look at some of these stamps and find a four-cent or even a six-cent tax, you will be less disturbed over the cost of drugs today. The claims made for some of these products were stupendous. It was because of some of these claims that I became interested in trying to find out what the preparations contained and I discovered a field of investigation that was amazing and amusing.

Roughly, this period of time is only eighty to ninety years ago and I can look back over at least more than half of that time and recall that the use of some of these preparations was carried over into the earlier 1900 days. I visualize some of the ads in the newspapers, especially one which described the virtues and benefits of Mrs. Allen's Hair Restorer. The newspaper ad sometimes displayed the same cut as is pictured on this stamp, but there was also one of this same lady taken from the rear showing her long, curled tresses floating down below the lumbar region. Mrs. Allen's Hair Restorer had a remarkable sale. In urban places it was well advertised. In the rural and midwest frontiers it was peddled by the medicine show men who played the small towns alone or accompanied a carnival group that made up the fairs in those isolated regions.

An account of one of these medicine shows appeared in one of the best sellers of a few years ago—Lockridge's *Raintree County*. The scene is a carnival. With a small crowd around him, the hawker begins his "spiel":

> It is nothing but a bottle, folks, a simple unadorned bottle and yet, friends, this plain, simple and ordinary bottle contains in it a secret preparation. The miracle worker of our age. Ladies and Gentlemen, may I have a little of your precious time to describe the extry ordinary virtues of the elixir contained in this bottle. You will permit me to indulge in a personal reminiscence.
>
> [There follows a description of his own hairlessness. Then he continues.]
>
> The Lord never does anything without a purpose, and if he meant mankind to go about with his skull naked of hair, why did he bestow on us this lush and luxurious foliage that in our natural state starts and stands triumphantly according to the words of the poet. With all its fronds in air, Fellow Americans, the good Lord intended [again he digresses into a reference to his hairlessness until someone recommended Mrs. Allen's. He was skeptical at first but finally gave in. He adds, within a week or two, a noticeable change was apparent.] My hair began to recover the black lustre it had in my younger days when a boy in the hills of Western Virginia. My head became entirely clear of dandruff and new hair grew where the old had been. You see

FIG. 1. Some United States Revenue stamps used on nineteenth century patent medicines.

before you today a man whose pride and hair have been restored together and general health improved.

It was in 1914 that the Connecticut Agricultural Station made a chemical analysis of Mrs. Allen's Hair Restorer and found it was made up of a glycerine and water solution of lead acetate and some sulphur. This certainly cannot be recommended as a bathing mixture but if it had no other value, it might at least have helped to reduce pediculosis since the amount of hair women had in those days provided good breeding ground for nits. That Mrs. Allen's Hair Restorer had little value in treating baldness was discovered by the little boy who, as the story goes, after listening to the hawker was impelled by curiosity to peek under the back of the tent just as the man was removing his wig and mopping his sweating head.

The same hawker selling Mrs. Allen's ended his "spiel" with the following:

Free gratis and for no extree charge this large bottle of Dr. Hostetter's celebrated Stomach Waters, guaranteed to cure any or all diseases of the alimentary tract, nervous, respiratory and circulatory systems, to wit; stomachache, heartburn, dyspepsia, diarrhea, dysentery, dizziness, fainting spells, biliousness, piles, pimples, arthritis, lumbago, rheumatism, jaundice, kidney trouble, female complaints, and organic weaknesses caused by youthful indiscretion or the approach of old age.

Hostetter's bitters was an extremely popular remedy, because if you merely followed directions for its use, which were of course cautious and moderate, you still got the equivalent of the alcohol in two bottles of beer and you could keep your membership in the Temperance League. Of course, it wasn't necessary to follow directions; you could use more bottles and also larger ones. Many men kept two bottles on hand, one in the house and one in the barn in order to meet any emergency. In my collection there are a number of Hostetter stamps, including a two-and-a-half and a four-cent tax fee size. Here, undoubtedly, was the beginning of the economy size package.

Hostetter's was about 75 per cent alcohol; other bitters contained similar amounts and were thus popular remedies of the day, for alcohol, while generally used, was also often frowned upon. One bitters company made use of the delightful slogan "Nil Desperandum." I also have a stamp rarity —Lippman's Bitters—only eighteen copies being known to exist. In a recent historical novel of the life of Mary Todd Lincoln there are several references to the medicine men in the market places plying their wares, among these were undoubtedly the favored tonic bitters.

Another popular remedy was Mrs. Winslow's Soothing Syrup. This stamp shows a cretin-appearing baby holding a doll. This remedy was sup-

posed to be a good one for teething, summer colic, and a number of other infant ailments. It was made up of opium, morphine, sodium and potassium bromide, alcohol, an essential oil, with anise and sugar to flavor it—a genuine Mickey Finn which undoubtedly did soothe those to whom it was administered. This remedy could well be labelled the No. 1 Baby Killer of its age. It was widely sold here and in England and eventually was barred from sale first abroad and then in this country.

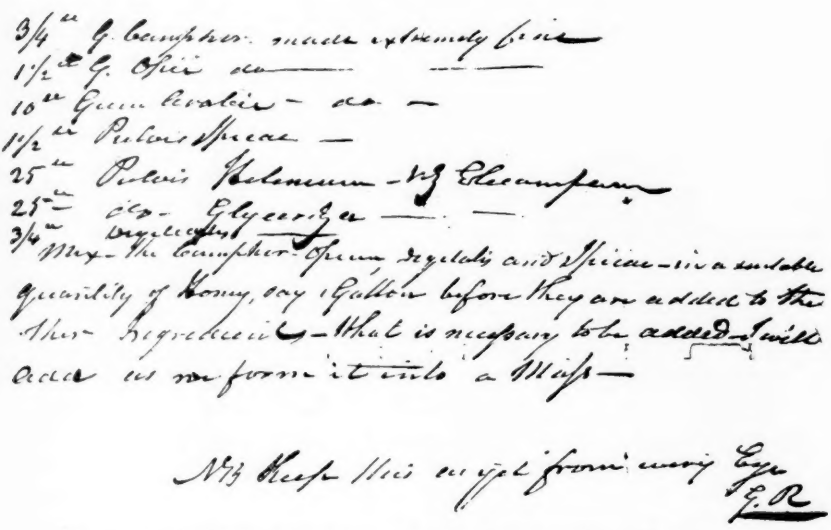

FIG. 2. A typical formula for a patent medicine. The admonition "Keep this receipt from every eye," or something similar, was usually included.

One of the drug companies of that period was located in East Hampton, Connecticut. At that time the population of the village was fifteen hundred persons. The company did such a thriving mail order business that it gave the village a second-class post office rating. This company put out a remedy that was supposed to conquer kidney and bladder conditions as well as rheumatism and other ailments when due to excessive uric acid. I suppose the suggestive influence helped the person to conclude that his uric acid was perhaps excessive in the same way that today people become obsessed with the idea that their sterates need fixing. The remedy was made up of oil of wintergreen, some potassium acetate, and some bicarbonate. It prob-

ably would not matter much whether you rubbed the stuff in or took it internally.

Another famous remedy for urinary tract diseases was Dr. Pierce's Golden Medical Discovery. This man made up his pills in the shape of a kidney and colored them red. This pill was a real charge of buckshot. It contained acetate, chloride, carbonate, iodine, phosphate, and salicylate; also ammonia, calcium, iron, potassium, sodium emodin, alloin, hexamethalinamine, and sugar. You can see it was bound to cure something. This famous Dr. Pierce, whose fading picture you can still see on some of the barns throughout the country, also gave out a small sample of something that was even better called Dr. Pierce's own Prize Remedy. The things that this was good for included almost every known ailment, but it was especially valuable in every kind of female trouble. In fact, it could raise the womb itself. The testimonials in regard to this remedy were amazing and naturally were distributed in every package.

In those days, the medicines that sold best were those purported to be good for many illnesses. Families were being practical when they bought such a remedy. Witness, for example, Dr. A. W. Chase's famous nerve pills which were indicated for watery blood, nervous disorders, brain fog, nervous headache, nervous dyspepsia, irregular heart action, sleeplessness, sallow complexion, paralysis, and locomotor ataxia. This sounds like the complaints of most of my patients. These pills contained nothing but sulphate, carbonate, iron, manganese, and some alkaloids, and from my experience these ingredients do not cure the above conditions. There was, however, one other element, an extract made from a very special bean called the Saint Ignatius Bean which was alleged to have been originated by the Reverend John W. Dagnal in 1854. The bean must have been lost, but if I could only get hold of that bean, it would eliminate all the need for barbiturate and benzedrene. I could cure watery blood and brain fog and insomnia and charge more than the astrologers. One has only to look at the picture of Dr. Chase, at his luxurious beard, his kindly, dignified, and calm face to imagine that people just looking at his picture probably figured he took the stuff himself and so rushed to buy.

Another drug with wide claim of usefulness was John Bull's Sarsaparilla Compound. This was good for erysipelas, chronic sore eyes, ringworm, tetter, scald, and dropsy. It was made up of potassium iodide, some vegetable extract, and sarsaparilla. It probably wasn't bad tasting and it is unfortunate that it wasn't converted into a soft drink.

Some companies put out products which were very rational. For example, Kidder's Cold and Grippe Tablets. They consisted of phenacetin, salol,

caffeine, and citric acid. That is not far removed from some of our prescriptions today. Compare them, however, with Dr. Schenck's Pulmonic Syrup which was nothing but a wintergreen-flavored mixture of saccharine syrup, 96 per cent sugar, and vinegar of squill. It sold for $2.50 a quart and was no better than pure molasses, which at that time sold for 50 cents a gallon. Another of similar nature was Piso's Consumption Cure. It was made up of alcohol, chloroform, and cannabis indica. Piso had another prize package, namely, tablets good for female regulation—apparently a very prevalent ailment of that day because so many companies put out concoctions for female troubles. Dr. Cheeseman's Female Regulator was made up of aloes and ferrous sulphate. There is no doubt that something was regulated.

Radways Sarsaparilla claimed to cure cancer, falling of the womb, paralysis, diabetes, consumption, and leukorrhoea. Its content was an alcoholic solution of sugar, potassium iodide, arsenic, and alkaloid. This drug was still being sold in 1918 when the company was brought into court and convicted of fraudulent advertising. The R. G. Wright Company, manufacturers of Wright's Indian Vegetable Pills, was likewise convicted of fraudulent advertising. These pills were said to cure yellow fever, erysipelas, consumption, cancer, venereal disease, paralysis, epilepsy, and other conditions.

A rather common stunt used in the advertising of some of these products was an absolute money back guarantee if, after taking three quarters of the bottle with no improvement, you returned the bottle. There is something about the human being, though, that makes him wary, so after patiently taking most of the bottle he debates with himself and then proceeds to finish it. Thus very few companies ever paid any refund.

Another big hoax of the time was that of Collins Voltaic Plasters. These plasters were said to relieve pain and inflammation of the kidneys, were valuable in fevers and ague, indigestion, bilious colic, affectations of the liver, bone fractures, bronchitis, and asthma, and gave gratifying relief in female weaknesses. This panacea required a four-cent tax stamp. I suppose one had to get the large-sized box when you consider how much of one's anatomy one had to cover. It was similar plasters that inspired Mark Twain to write the lines, "Alcocks Patent Porous Plaster makes all pain and ache fly faster."

There is another item of interest about these plasters, i.e., the name Voltaic. This was a period in which electrical industry was being developed, therefore one finds the names voltaic, galvanic, magnetic, faradic—terms comparable to our use of atomic today. These terms connoted power, force, virility. A number of salves were so named, as is illustrated by "Dalleys

Galvanic Salve" which had the added inference of potency in the slogan "Good for Man and Beast." Naturally, if a salve was good for a beast, it must be potent and people looked with favor on its alleged strength.

There was also Radways Ready Relief, a product good for rheumatism, pleurisy, pneumonia, headache, burns, scalds, spinal affections, fevers, dysentery, cholera, and paralysis. It was made up of an oleoresin of capsicam (red pepper), camphor, and ammonia. I can actually see the stomach mucosa writhe with every swallow. It is an example of what would be considered potent.

Most appealing to me was the product of Dr. William's Company: Pink Pills for Pale People. What a beautiful slogan! No vitamin or hormone of today is so delightfully advertised. The pill itself was 37 per cent sugar with some iron sulphate, potassium carbonate, and starch, with just a trace of strychnine. Accompanying each box was a most explicit set of directions, including proper dietary measures, walks and exercises, hot and cold baths, and right thinking, which, if a person could have followed it, was a regime redolent with so much health and virtue that I doubt if he could have lived with himself, much less his neighbor.

There was the trend, too, to appeal to the whole household as illustrated in Dr. Harter's Family Medicines. His "Little Liver Pill" was illustrated pictorially by two pictures. One little boy sitting on a pottie obviously uncomfortable and the other likewise on a pottie and smiling and the caption underneath "He took his little liver pill." Dr. Clements C. Fry has this picture in his collection of medical Americana.

There were also products named Father Matthews, Father Johns, etc., the implication being that the medicine was developed by someone in the priesthood. The use of the implied religious origin produced a definite stimulus to sales. This serves as a good illustration of the continued interweaving of medicine and religion from much earlier times. Actually, Father Matthew was probably a layman who would spend several days in the woods pulling up sassafras roots and boiling his concoctions in the back woodshed. I have in my collection a letter from such a man, writing to a banker in which he agrees that he will gather enough roots and leaves to guarantee a constant supply and wants the banker to underwrite the cost of the bottles. Another letter shows an awareness of the aesthetic values to be considered, for the writer says at the conclusion of a recipe for a salve, "I prefer to make it a flesh colour—*Keep this to yourself.*"

You will also find pictures of youth leading a stallion—Young America, virility and strength being implied in picture and slogan. And consider Galvanic Salve, Mexican Mustang Liniment, Centaur Ointment—the

strength of bulls mixed with the touch of mysticism. One stamp shows a man with a machette attacking a skeleton, the stark symbol of death. Another stamp shows a man wearing a liver pad hung around his neck. Usually these pads were filled with asfoldita, which was alleged to ward off disease. The more I think of it, the more I wonder if it might not have had its value because people naturally steered clear of the odor.

I should like to mention how some of these products were distributed. After all, production is only half the battle. In this period many of these pills and concoctions were distributed by pack trains and by the peddler. For example, the makers of Soule's Sovereign Balm of Life Pills, manufactured in upper New York State, sent their boxes to Buffalo and thence by boat to Cleveland. This was the terminus of the pack peddler who with his wagon cart covered the territory of Ohio, Indiana, and even into Illinois and Michigan, dropping off packages as he went through the little towns and picking up his money on his return trip.

Others used similar methods, but one of the cleverest devices was that employed by the Burdsell Company of Cincinnati, Ohio who manufactured the famous "Scandinavian Blood Pills." This company used a ship and plied the eastern seaboard, doing a thriving business on the docks, the inference being that they had just arrived from Scandinavia with a fresh supply. This company even had the audacity to illustrate their stamp with the picture of a buccaneer as the central design. They were pirates, indeed.

Today we have aureomycin, streptomycin, chloromycin, and a host of other icings. We have ACTH and cortisones, vitamins and hormones. We have made medical practice more specific to the ailment. We perhaps are a healthier generation. I doubt, however, whether we are any happier. For sickness still has many values. It is the only socially acceptable reason for not having to put forth effort. The fact still remains that "man likes to take things." He wants his problems erased, not corrected, and he will still fall for almost anything that offers him a quick and guaranteed cure.

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STUDIES ON THE METABOLISM OF PLASMA PROTEINS IN TUMOR-BEARING RATS‡

INTRODUCTION

The "autonomy" of neoplastic tissue is intimately related to its ability to carry out net synthesis of protein regardless of the demands of the host. Clinically, this is manifest in the failure of the tumor to exhibit the wasting of other tissues of the cachectic host. Accordingly, Mider and others[18] have referred to tumors as "nitrogen traps." In experiments of LePage *et al.*[19] on this subject, the weight of most nontumor tissues of fasted tumor-bearing rates diminished to 30-50% of values for control tissues. On the other hand, the increase in weight of tumors was not significantly different in fasted animals and those fed *ad libitum*. Moreover, the uptake of C^{14} in proteins of the tumors was equal in rate and quantity in fed and fasted tumor-bearing rats following injection of glycine-2-C^{14}.

Among the possible explanations for these clinical and laboratory findings, two have been investigated recently, namely, the possibility that tumors might have a peculiarly great avidity for amino acids of the blood and the possibility that tumors might take up plasma proteins directly. Initial evidence that tumors exhibited a greater uptake of amino acids came from the studies of Zamecnik *et al.*[23] who reported that hepatoma slices incorporated isotope of labeled alanine into protein at a rate eight times that of slices of normal liver or liver of the hepatoma-bearing rat. These findings have been confirmed by Winnick *et al.*,[22] but they are in sharp contrast to the results obtained after injection of labeled amino acids into tumor-bearing rats. In 1944 Shemin and Rittenberg[17] found that uptake of N^{15}-labeled amino acids into protein *in vivo* was not greater in tumors than in other tissues and their findings were subsequently confirmed for a variety of tumors by Zamecnik *et al.*,[24] Norberg and Greenberg,[14] and others; the

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‡ This investigation was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research and an Institutional Grant from the American Cancer Society.

Received for publication January 21, 1955.

present studies provide further confirmation of the results of Shemin and Rittenberg.

Utilization of plasma protein as a source of amino acids was demonstrated by Whipple and Madden who infused plasma into fasted dogs and maintained their nitrogen balance.[21] In the normal animal, the rate of turnover of plasma proteins is rapid, as is indicated by the turnover time of 8.2 days for albumin in the rat.[19] While these findings have indicated that plasma proteins are utilized by nontumor tissues, evidence has only recently been presented for concentration of plasma protein in tumors. Korngold and Pressman[11] reported localization of antibodies labeled with I^{131} in the Murphy-Sturm lymphosarcoma and Wagner[20] has found that virus in tumors is destroyed as the antibody level of the host rises. Babson and Winnick[1] noted a greater uptake of isotope of biosynthetically-labeled plasma proteins by tumors than by liver and kidney. They found that the relative specific activities of two labeled amino acids was the same in plasma proteins and proteins of tumors and, further, the labeling of tumor protein was not affected by injection of large quantities of non-radioactive amino acids.

Our interest in this subject has developed from two lines of investigation. In studies on the metabolism of acetate-1-C^{14} and pyruvate-2-C^{14} in tissues of tumor-bearing rats, it was found that nontumor tissues rapidly transferred the isotope to amino acids. In experiments with acetate-1-C^{14}, the isotope in nontumor tissues was found chiefly in glutamic acid and in experiments with pyruvate-2-C^{14}, the isotope in nontumor tissues was found chiefly in glutamic acid and alanine.[2,3,4] On the other hand, the tumors studied did not metabolize acetate-1-C^{14} and transferred the isotope of pyruvate-2-C^{14} primarily to lactate. These experiments suggested that the tumors might be dependent upon other tissues for their supply of "non-essential amino acids" for protein synthesis.

Another suggestive line of investigation[9] concerned transplantation experiments involving the Shope papilloma. It was found that adult rabbit skin infected *in vitro* with the Shope virus and transplanted to the brains of immune rabbits developed papillomas containing infectious virus. In like manner, the infectivity of the virus remained unchanged in domestic rabbit papillomas reinfected *in vitro*, despite growth in immune animals. In contrast, no evidence of virus could be found in the V2 carcinoma (the malignant tumor derived from the Shope papilloma) after similar treatment although infectious virus could be recovered after growth in normal nonimmune rabbits. These results suggest that antibody failed to penetrate the cells of normal skin or of benign papilloma but did penetrate the cells of

the carcinoma and indicate that the transformation to cancer was associated with an increased permeability of cell walls.

The experimental questions which arose from these studies were: (i) could tumors take up plasma protein to a greater extent than other tissues, and (ii) could the tumors utilize these proteins for growth?

MATERIALS AND METHODS

The animals used in these experiments were male Sprague-Dawley rats weighing 150-200 grams. They were obtained from the Charles River Laboratories in Boston, Massachusetts, and were fed *ad libitum* on Purina Laboratory Chow. The Walker 256 carcinosarcoma and Jensen sarcoma used in these experiments were transplanted by Mrs. Rose K. Busch. The tumors were transplanted 7-9 days prior to the experiment and weighed 3-5 grams in the aggregates of 2-4 sites of transplantation.

Glycine-2-C^{14} and DL-lysine-2-C^{14}, with specific activities of 19 microcuries per mg. and 3.3 microcuries per mg. respectively, were obtained from Tracerlab, Inc. In studies on the uptake of radioactive amino acids, 0.5 mg. of labeled glycine or 1 mg. of labeled lysine* was injected intraperitoneally into tumor-bearing rats. For preparation of the radioactive plasma, 2 mg. of labeled glycine or 10 mg. of labeled lysine were injected intraperitoneally into normal rats weighing 250-350 grams. At the end of the desired interval, generally three hours, the rats were anesthetized with nembutal (0.5 mg./100 grams body weight) and exsanguinated by arterial puncture at the bifurcation of the aorta by means of a 20 ml. syringe and a 19-gauge needle; 0.25 ml. of heparin (5000 U./ml.) were added to the syringe. The heparinized whole blood was centrifuged at 1500 r.p.m. for 10 minutes and the supernatant plasma was dialyzed for a minimum of two hours against a buffer of 0.1 M glycine or DL-lysine and 0.01 M sodium phosphate buffer at pH 7.4. An average of 375,000 c.p.m. (counts per minute) was found in 5 ml. of plasma and the average specific activity of the plasma protein was 1100 c.p.m./mg. At three hours after intravenous injection of 3-5 ml. of the radioactive plasma into tumor-bearing rats, the average specific activity of plasma proteins of the recipient rats was 280 c.p.m./mg.

At the selected time, the tumor-bearing rats receiving the radioactive tracer were anesthetized with nembutal and exsanguinated as noted above. The tissues were excised and placed in liquid air[5] except in experiments in which the cellular particulates were isolated by differential centrifugation. Following dispersion of the tissues in perchloric acid, the precipitated proteins were treated with 5% trichloroacetic acid, 95% ethanol, ethyl ether, chloroform-methanol (2:1) twice,[18] petroleum ether-acetone (1:1), and butanol. The suspension of protein in butanol was placed on an alundum disk in a separated glass funnel on a filter flask connected to a vacuum line; suction was applied until the plate was dry. Radioactivity was determined in a preflush flow counter.

In experiments involving differential centrifugation of the homogenates, the excised tissues were placed in ice-cold saline and then dispersed in 0.3 M sucrose. The procedures used in preparation of nuclear and mitochondrial fractions were essentially

* The authors are indebted to Dr. D. M. Bonner for initial samples of these amino acids.

those of Schneider[16] and were carried out in a model PR-2 International refrigerated centrifuge. The microsomal fraction was prepared by centrifugation of the sample in a Spinco preparative centrifuge in an average gravitational field of 60,000 g for one hour.[10]*

RESULTS

Figure 1 presents the rates of labeling of proteins of liver, Walker 256 carcinosarcoma, kidney, Jensen sarcoma, and skeletal muscle following injection of glycine-2-C^{14}. These data are in agreement with those of previous reports[14, 17, 24] which have indicated that uptake of isotope of amino acids

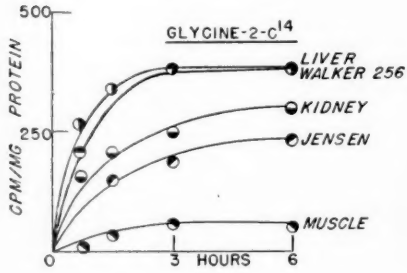

FIG. 1.

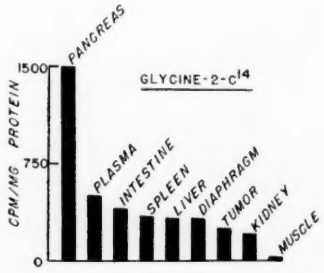

FIG. 2.

FIG. 1. Rates of labeling of tissue protein following injection of glycine-2-C^{14}. Data are averages of two to four experiments carried out at each time point.

FIG. 2. Specific activities of protein of tissues three hours after intraperitoneal injection of glycine-2-C^{14}. Values for the Jensen sarcoma and Walker 256 carcinosarcoma are pooled. Specific activities of protein of heart, testis, thymus, and brain ranged between those of muscle and kidney.

by protein of tumors is not significantly different from that of nontumor tissues. While the increase in specific activity of protein of the rapidly growing Walker 256 carcinosarcoma was equal to that of liver, values for protein of the Jensen sarcoma, a more slowly growing tumor, were lower than those of liver or kidney. The curves reached a plateau in the 3-6-hour interval. The specific activity of proteins of skeletal muscle was very low throughout the study. Figure 2 presents specific activities of protein of various tissues three hours after injection of glycine-2-C^{14}. It is apparent that the values for the tumors are within the range for those of other tissues. The specific activities of protein of the spleen, plasma, whole intestine and pancreas were all significantly greater than those of the

* We are indebted to Drs. D. M. Bonner and J. H. Milstone for the use of their Spinco ultracentrifuges.

tumors at this time. It is of interest that Friedberg *et al.*[8] have shown that values for intestinal mucosa were much higher than those of kidney, liver, or plasma, and slightly higher than pancreas. The specific activities of proteins of the heart, testis, thymus, and brain were determined at three hours after injection of the labeled glycine and these values ranged between those of muscle and kidney (Fig. 2). In studies with DL-lysine-2-C^{14}, essentially the same findings were obtained three hours after injection of the tracer. The specific activity of protein of the Walker 256 carcinosarcoma approximated that of liver or spleen (Fig. 1), while the specific activity of protein

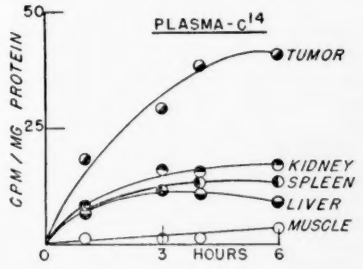

FIG. 3.

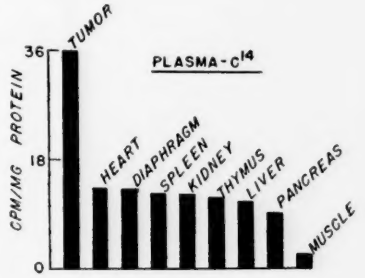

FIG. 4.

FIG. 3. Rates of labeling of tissue protein following injection of labeled plasma. Values for the Jensen sarcoma and Walker 256 carcinosarcoma were pooled inasmuch as significant differences were not found. The specific activity of the plasma proteins of the recipient rats varied from 180-330 c.p.m./mg. with an average value of 280 c.p.m./mg.

FIG. 4. Specific activity of protein of tissues three hours after injection of plasma labeled with glycine-2-C^{14}. Values for Walker 256 carcinosarcoma and Jensen sarcoma are pooled. Specific activities of protein of intestine, brain, testis, and lung ranged from 1.7-8.2 c.p.m./mg. and fall between those of muscle and pancreas.

of the pancreas was three to four times that of protein of the spleen (Fig. 2). Values for lung, kidney, and muscle decreased in the same order as with glycine-2-C^{14}.

PLASMA PROTEIN

The rates of uptake of isotope of plasma protein by protein of tissues is indicated in Figure 3. The specific activity of protein of the tumors increased steadily in the first four hours after injection of the labeled plasma; no significant differences were found in the data for the Jensen sarcoma and the Walker 256 carcinosarcoma. The rate of increase of the specific activity of protein of the tumors was 2½-10 times that of other tissues (Fig. 3).

The curves for kidney and spleen reached a plateau in the 3-6-hour period, while that for liver began to decline after the third hour. The specific activity of protein of skeletal muscle was very low at each time point studied. At three hours after injection of the labeled protein, values for the specific activity of protein of the tumor were 35-41 c.p.m./mg. (Fig. 4) compared with a range of 11-14 c.p.m./mg. for heart, diaphragm, spleen, kidney, thymus, and liver. Values for intestine, brain, testis, and lung ranged from 1.7-8.2 c.p.m./mg. and would appear in Figure 4 between pancreas and

TABLE 1. LABELING OF INTRACELLULAR PARTICULATES FOLLOWING INJECTION OF LABELED PLASMA

Values are c.p.m./mg. protein of the particulates and supernatant solutions. Methods of preparation of microsomal and mitochondrial fractions are in the text. Each value in the table is the average of four experiments, two with glycine-2-C^{14} labeled plasma and two with DL-lysine-2-C^{14} labeled plasma. Values are pooled inasmuch as no significant differences were found for the experiments with the two labeled amino acids. Ranges are presented in parentheses.

Tissue	*Whole homogenate*	*Mitochondrial fraction*	*Microsomal fraction*	*Supernatant fraction*
Walker 256 carcinosarcoma	32.4 (21.8-43.6)	26 (20.2-36.9)	29.5 (18.4-46.0)	54 (22.4-77.0)
Liver	11.2 (10.0-11.8)	11.5 (7.4-17.6)	10.5 (9.3-11.5)	25 (8.7-49.2)
Kidney	13.1 (10.5-14.7)	8.6 (6.4-9.7)	9.1 (6.8-10.2)	41 (14.1-39.7)
Lung	58.8 (27.4-95)	26 (9.2-45)	11.1 (6.7-18)	99 (52.4-146)

muscle. The specific activities of protein of the lung varied markedly, i.e., from 11 c.p.m./mg. to 75 c.p.m./mg., and were occasionally highest of the tissues studied. This variation may be due to the relatively large amount of blood or plasma pooled in the very small lung tissue mass or the uptake of protein aggregates by lung. In agreement with the values obtained for plasma labeled with glycine-2-C^{14}, the specific activity of protein of the Walker 256 carcinosarcoma was two to three times that of liver or kidney, three hours after injection of plasma protein labeled with DL-lysine-2-C^{14}.[1]

LABELING OF CELLULAR PARTICULATES *IN VIVO*

The possibility that isotope of plasma protein entered the protein of intracellular particulates was investigated by determination of the specific activity of proteins of these particulates after tissue homogenates had been

subjected to differential centrifugation.[30, 16] Table 1 presents the specific activities of proteins of cellular particulates of Walker 256 carcinosarcoma, kidney, liver, and lung. Values for the nuclear fractions are not presented inasmuch as they closely reflected values of the whole homogenates; in these experiments no special precautions were taken for purification of the nuclei. The specific activities of proteins of the mitochondrial, microsomal, and supernatant fractions were sufficiently similar in experiments with either labeled glycine or lysine that the data were pooled in Table 1. The

TABLE 2. LABELING OF INTRACELLULAR PARTICULATES BY INCUBATION WITH LABELED PLASMA

In these experiments, 0.5 and 1.0 ml. of labeled plasma were incubated with homogenates of tissues over a range of times with no significant changes in the labeling. Values presented are specific activities of proteins of particulates and supernatant solutions.

Tissue	*Ml. labeled plasma*	*Whole homogenate*	*Mitochondrial fraction*	*Microsomal fraction*	*Supernatant fraction*
Tumor	0.5	106	39	70	350
	1.0	264	78	154	508
Liver	0.5	66	38	37	166
	1.0	161	82	103	340
Kidney	0.5	210	49	59	818
	1.0	345	90	103	...
Lung	0.5	390	119	43	930
	1.0	660	95	207	880

data for mitochondria reflect the same trends as those for microsomes with the exception that the average value for lung was equal to that for tumor, although the variation was very great. The control experiments for these studies are presented in Table 2. Inasmuch as the labeling of protein of the particulates could have occurred by adherence of plasma proteins to mitochondrial and microsomal preparations or by precipitation of particles, such as small clumps of fibrin, from plasma in the same gravitational field as the cellular particulates, it was necessary to determine the extent of these processes before a conclusion could be reached on the incorporation of plasma proteins or their degradation products into the particulates.* Accordingly, varying amounts of labeled plasma were incubated with homogenates of liver, kidney, lung, and tumor for varying intervals, and

* We are indebted to Dr. G. A. LePage for helpful suggestions on this point.

the specific activity of the cellular particulates was determined as in the *in vivo* experiments (Table 2). From these studies, ratios could be obtained for the relative specific activities of proteins of the particulates and those of the supernatant fraction. In tumors, the ratio of the specific activity of protein of the mitochondrial fraction to that of the supernatant fraction varied from 1:7 to 1:9, and the corresponding values for the microsomal fraction were 1:3.5 to 1:5 in the *in vitro* studies. Since the specific activity of protein of the supernatant fraction of the tumor was 54 c.p.m./mg. in the *in vivo* experiments (Table 1), the maximal *in vitro* labeling which could occur if all the isotope were in plasma protein would be 6.7 c.p.m./mg. for protein of the mitochondria and 13.5 c.p.m./mg. for protein of the microsomes. Accordingly, the minimal extent of labeling *in vivo* was 19.3 c.p.m./mg. for protein of tumor mitochondria and 16 c.p.m./mg. for protein of tumor microsomes. For the mitochondria of liver, kidney, and lung, the corresponding values were 5.7, 4.6, and 14.4 c.p.m./mg., respectively. For the microsomes, the corresponding values were 4.8, 4.1, and 3.5 c.p.m./mg. for liver, kidney, and lung, respectively. It would thus appear that labeling of the protein of the mitochondria of tumors was 3.5 and 4.2 times that of liver and kidney, respectively, while the labeling of microsomes of tumor was 3.5 to 4.8 times that of liver, kidney, and lung. These data suggest that the tumor is not only more effective in trapping radioactive plasma, but is also more effective than other tissues in its utilization.

DISCUSSION

These initial experiments indicate a greater labeling of "tumor protein" than proteins of other tissues following injection of radioactive "plasma." It is clear that both "tumor protein" and "plasma" represent complex mixtures of proteins and other compounds. From the studies of Cohn and his co-workers,[6] it is evident that there are many kinds of proteins in plasma and undoubtedly even the number of different antibodies is large. In experiments now in progress, radioactive plasma proteins are being separated by the methods developed by Cohn *et al.*[6] and the isolated fractions are being reinjected into tumor-bearing rats. It remains to be determined whether one or many protein fractions contribute to the labeling of proteins of the tumor. On the other hand, "tumor protein" is a mixture of compounds which share the properties of insolubility in protein precipitants and organic solvents used for extraction of lipids. The nature of the proteins which are the recipients of the isotope of radioactive plasma has not been studied, but it seems possible that only a part of the proteins of the tumor can be synthesized from products of plasma proteins. In this case, an extensive study

of the labeling of individual proteins of the tumor would be justified. Before an understanding of the mechanism of utilization of the plasma proteins can be achieved, specific proteins of tumors will have to be isolated and degraded. These studies do not permit evaluation of the extent of degradation of plasma protein before the fragments are incorporated into tumor protein.

While the possibility exists that the avidity displayed by the tumors for plasma protein reflects a permeability difference between tumors and other tissues,[7] either at the cellular level or at the level of tissue structure, the evidence presented indicates that there is transfer of isotope to cellular particulates *in vivo*. Moreover, it is possible to calculate that 1000 mg. of tumor protein could take up 1030 mg. of plasma protein in 24 hours, an amount sufficient to account for the known doubling in weight of the tumor in this time interval.[12] Thus, in three hours, the specific activity of protein of the tumor was 36 c.p.m./mg. so that one gram of tumor protein would take up 3.6×10^4 c.p.m. in three hours. Since the specific activity of plasma protein of recipient rats averaged 280 c.p.m./mg., 3.6×10^4 c.p.m. represents an uptake of 128 mg. of plasma protein in three hours. If this rate were constant, one gram of tumor protein would take up 1030 mg. of plasma protein in 24 hours. These considerations are based on values for the whole homogenates. Although similar calculations for the uptake of amino acids are difficult because of the unknown size of the pool diluting the radioactive precursor, it is possible to calculate the percentage of isotope of glycine-2-C^{14} or lysine-2-C^{14} injected which enters the tumor protein. Of the 11,000,000 c.p.m. of labeled amino acid injected, 200,000 c.p.m. can be accounted for in 500 mg. of tumor protein (average specific activity equals 400 c.p.m./mg.). This value represents 1.8% of the total amino acid injected. On the other hand, 18,000 c.p.m. of 375,000 c.p.m. in the injected plasma protein were found in the tumor. At three hours, an average of 245,000 c.p.m. remain in the plasma and accordingly the tumor has removed 12.8% of the isotope lost from the plasma.

These experiments may be of significance in three aspects of the study of neoplasia. First, the growth of the tumor in the starving animal and cachectic human may be related to its ability to trap plasma protein and utilize the degradation products for synthesis of tumor protein. Secondly, the ability of the tumor to invade and metastasize may reflect its more favorable nutritional requirements or conversely, the ability of the tumor to take up label of plasma protein may reflect cellular changes important to invasion and metastasis. Finally, the factors involved in concentration of

the plasma protein may be related to differences between benign and malignant tumors in that the former may not effectively take up plasma proteins.

SUMMARY

1. The rate of uptake of isotope of glycine-2-C^{14} and DL-lysine-2-C^{14} by proteins of tumors was in the range for nontumor tissues over the period of 45 minutes to 6 hours following intraperitoneal injection of the isotope.

2. The rate of uptake of isotope of plasma proteins labeled with glycine-2-C^{14} or DL-lysine-2-C^{14} by tumor protein was 2½ to 10 times that of other tissues studied, with the exception of lung which gave variable results.

3. Labeling of microsomal fraction of tumor was 3.5 to 4.8 times greater than that of kidney, liver, and lung three hours after intravenous injection of labeled plasma protein.

4. Labeling of the mitochondrial fraction of tumors was 3.5 to 4.2 times greater than that of kidney and liver respectively three hours after intravenous injection of labeled plasma protein.

5. It can be calculated from the data on labeling of tumor protein that uptake of plasma protein could account for the doubling of the weight of tumors in 24 hours.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Miss Catherine Joan Even.

REFERENCES

1 Babson, A. L. and Winnick, T.: Protein transfer in tumor-bearing rats. Cancer Res., 1954, *14*, 606.

2 Busch, H.: Studies on the metabolism of acetate-1-C^{14} in tissues of tumor-bearing rats. Cancer Res., 1953, *13*, 789.

3 Busch, H.: Studies on the metabolism of pyruvate-2-C^{14} in tumor-bearing rats. Unpublished.

4 Busch, H. and Baltrush, H. A.: Rates of metabolism of acetate-1-C^{14} in tissues *in vivo*. Cancer Res., 1954, *14*, 448.

5 Busch, H., Hurlbert, R. B., and Potter, V. R.: Anion exchange chromatography of acids of the citric acid cycle. J. Biol. Chem., 1952, *196*, 717.

6 Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Deroaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Lui, C. H., Mittelman, D., Mouton, R. F., Schmid, K., and Uroma, E.: A system for separation of the components of human blood. Quantitative procedures for separation of protein components of human plasma. J. Am. Chem. Soc., 1950, *72*, 465.

7 Duran-Reynals, F.: Studies on the localization of dyes and foreign proteins in normal and malignant tissues. Am. J. Cancer, 1939, *35*, 98.

8 Friedberg, F., Tarver, H., and Greenberg, D. M.: The distribution pattern of sulfur-labeled methionine in the protein and free amino acid fraction of tissues after intravenous administration. J. Biol. Chem., 1948, *173,* 355.

9 Greene, H. S. N., unpublished data.

10 Hogeboom, G. H., Schneider, W. C., and Streibach, M. J.: Localization and integration of cellular function. Cancer Res., 1953, *13,* 617.

11 Korngold, L. and Pressman, D.: The localization of antilymphosarcoma antibodies in the Murphy lymphosarcoma of the rat. Cancer Res., 1954, *14,* 96.

12 LePage, G. A., Potter, V. R., Busch, H., Heidelberger, C., and Hurlbert, R. B.: Growth of carcinoma implants in fed and fasted rats. Cancer Res., 1952, *12,* 1953.

13 Mider, G. B.: Some aspects of nitrogen and energy metabolism in cancerous subjects: A review. Cancer Res., 1951, *11,* 821.

14 Norberg, E. and Greenberg, D. M.: Incorporation of labeled glycine in the proteins of tissues of normal and tumor-bearing mice. Cancer, 1951, *4,* 383.

15 Potter, V. R. and Elvehjem, C. A.: A modified method for the study of tissue oxidations. J. Biol. Chem., 1936, *114,* 496.

16 Schneider, W. C., in *Manometric Techniques and Tissue Metabolism,* ed. by Umbriet, W. W., Burris, R. H., and Stauffer, J. F. Minneapolis, Burgess Publishing Co., 1949, p. 148.

17 Shemin, D. and Rittenberg, D.: Some interrelationships in general nitrogen metabolism. J. Biol. Chem., 1944, *153,* 401.

18 Sperry, W. M.: A method for the determination of total lipids and water in brain tissue. J. Biol. Chem., 1954, *209,* 377.

19 Ulrich, F., Tarver, H., and Li, C. H.: Effect of growth and adrenocorticotropic hormones on the metabolism of albumin in hypophysectomized rats. J. Biol. Chem., 1954, *209,* 117.

20 Wagner, R. R.: Influenza virus infection of transplanted tumors. I. Multiplication of a "neurotropic" strain and its effect on solid neoplasms. Cancer Res., 1954, *14,* 448.

21 Whipple, G. H. and Madden, S. C.: Hemoglobin, plasma protein and cell protein —their interchange and construction in emergencies. Medicine, 1944, *23,* 215.

22 Winnick, T.: Studies on the mechanism of protein synthesis in embryonic and tumor tissues. I. Evidence relating to the incorporation of labeled amino acids into protein structure in homogenates. Arch. Biochem., 1950, *27,* 65.

23 Zamecnik, P. C., Frantz, I. D., Jr., Loftfield, R. B., and Stephenson, M. D.: Incorporation *in vitro* of radioactive carbon from carboxyl-labeled DL-alanine and glycine into proteins of normal and malignant rat livers. J. Biol. Chem., 1948, *175,* 299.

24 Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L., and Steele, J. M.: Studies on the carbohydrate and protein metabolism of the rat hepatoma. Cancer Res., 1951, *11,* 592.

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POLIOMYELITIS ANTIBODIES IN TWO DIFFERING SOCIOECONOMIC GROUPS WITHIN THE SAME CITY‡

The study of the epidemiology of poliomyelitis has been complicated by the high ratio of inapparent to clinically recognizable infections and, until recently, by the lack of availability of practical laboratory methods to detect infected persons. A number of normal population groups have been surveyed for virus-neutralizing antibodies, which persist for many years, if not for life, and allow one to observe patterns of accumulated experience with poliomyelitis infections.[4, 6, 7, 9] Recently, tests for complement-fixing (C-F) antibodies have become available. These antibodies are relatively short-lived and thus are an index of relatively recent infection.[1, 2, 3, 8]

The present paper describes the first application of both neutralization and C-F tests to a survey of poliomyelitis infection in a city within this country, viz., Charleston, West Virginia. For this study, two population groups of differing socioeconomic status were selected. Antibodies against Type 2 poliomyelitis virus were measured in paired serum specimens collected in the spring and summer of 1951, and their age-specific prevalence considered in relation to environment and to the incidence of clinical poliomyelitis.

STUDY AREA

Two areas in Charleston were selected for study on the basis of obvious differences in the sanitary environment and socioeconomic status of the populations (see Fig. 1). The people in District 1 are a native white group of low to low middle socioeconomic status living in a physical environment which is grossly substandard. Human excrement is deposited in insanitary privies and in flush toilets with surface drained septic tanks. In 1951 there was no garbage collection. All blocks were littered with both organic wastes and assorted rubbish. District 4, located about three miles distant, is a clean

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‡ Aided by a grant from The National Foundation for Infantile Paralysis, Inc.
Received for publication February 11, 1955.

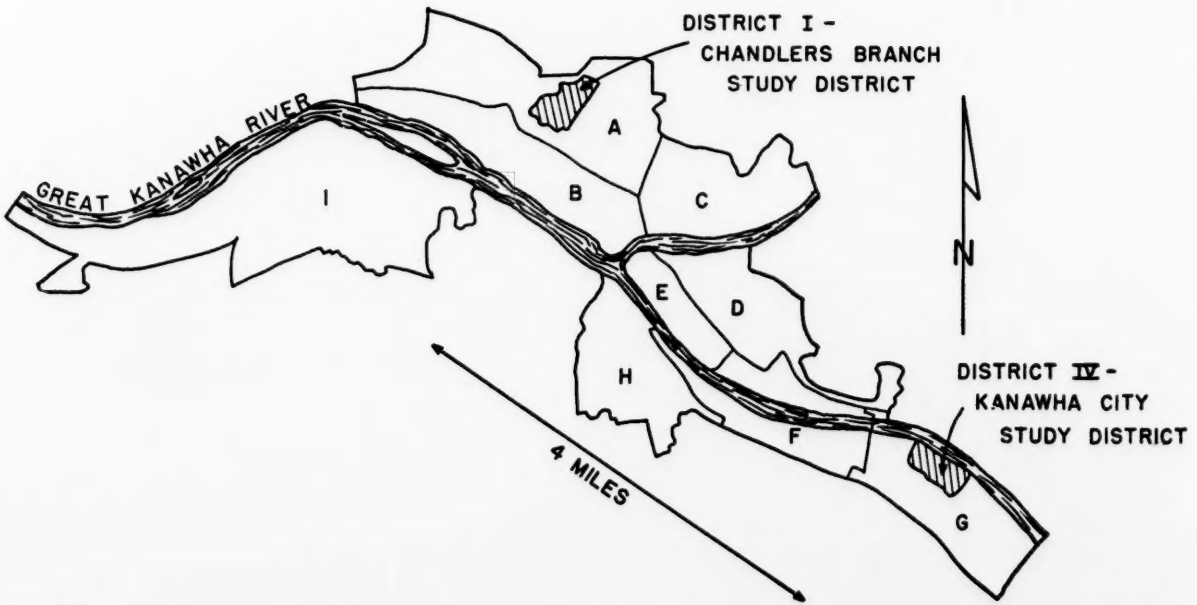

FIG. 1. Map of Charleston, West Virginia, showing the two study areas. District 1, low socioeconomic status, poor sanitary environment. District 4, middle to upper-middle socioeconomic status, high level of sanitation.

area, adequately sewered and conforming to accepted standards of environmental hygiene. The population varies from middle to upper middle class. The districts and the experience of the populations with Coxsackie virus infections in 1951 have been described.[10]

Prior to selection of the areas, in 1949-50, a sanitary survey of the city was made. Following selection of districts to be studied, trained interviewers obtained census data from an adult informant in each household and recorded observations regarding the socioeconomic status and sanitary environment of both individual premises and blocks. These observations were rechecked and changes noted at the time of monthly interviews to obtain morbidity data.

Specimens of blood were collected in May and in November of 1951 from a sample of the population in each district. The fall sample consisted of second specimens from as many of those bled in the spring as could be obtained, plus serum from new persons added to increase the numbers at ages under 15 years. Only households with children under 15 years of age were included. There was no other selection beyond the willingness of the families to co-operate with this phase of the study. In the spring over 80% of the households approached in District 1, and over 90% of those in District 4, joined in the study. In the fall, refusal rate of those bled in the spring was 12% in District 1 and 3% in District 4. Serum was separated within four hours of collection, and frozen and stored at —20° C. until examined in the laboratory.

LABORATORY METHODS

Neutralization tests. The procedure was the same as that used in earlier studies from this laboratory. In brief, 0.2 ml. of undiluted serum was mixed with an equal volume of Type 2 virus, Lansing strain, so that the final virus-serum mixture contained 100 LD_{50} per 0.03 ml., the volume inoculated in the brain of each test mouse. The mixture was allowed to stand at room temperature for one hour, and then was inoculated intracerebrally into eight mice. If six or more mice survived for a period of 21 days, the serum was recorded as positive for antibody. If no more than three mice survived, the serum was considered negative. With negative sera, usually all the mice died, with most of the mice showing paralysis before death. In doubtful cases, the tests were repeated.

Complement fixation tests. The specificity of the C-F reaction as due to infection with poliomyelitis virus is recognized, although type specificity is not as clear.[1, 2, 8] Crossing between types is common when large amounts of antigen are used with human sera, as was done in these tests. The procedure was again the same as that employed in an earlier study.[8] The Type 2 MEF1 strain which Casals had adapted to infant mice constituted the antigen.[1] Normal infant mouse brain antigens were included in each test. Before use, all sera were inactivated at 60° C. for 20 minutes. A modified Kolmer C-F test based on 100% hemolysis was used. Serum, antigen, and comple-

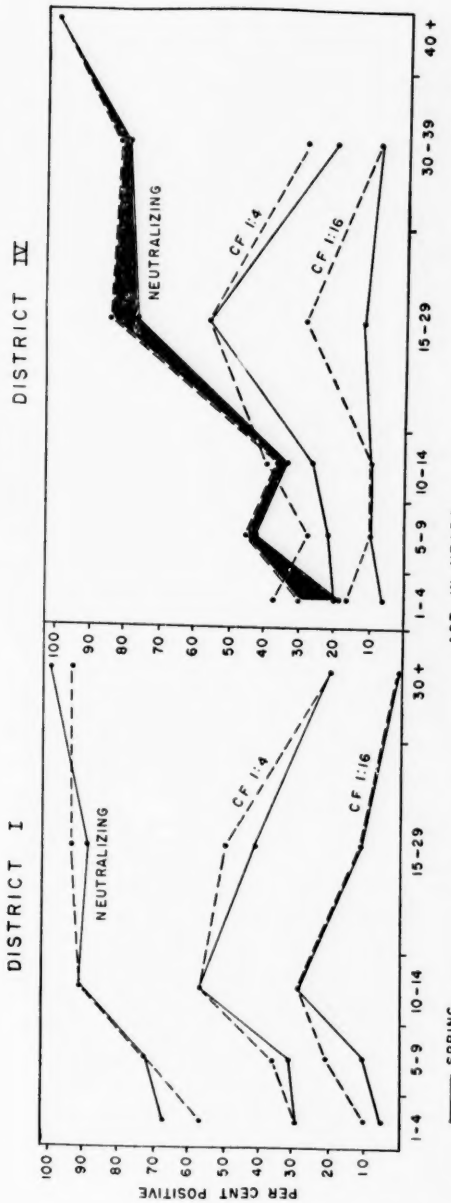

FIG. 2. Prevalence of neutralizing and C-F antibodies, at 1:4 and 1:16 dilutions, in paired sera in the spring and fall. Between 17 and 62 healthy persons make up each age group.

ment (2 units) were added in 0.1 ml. increments each, and after overnight incubation at 4° C., 0.2 ml. of a sensitized suspension of sheep red blood cells was added, the mixture kept for 30 minutes at 37° C. and read.

Each lot of antigen was tested for antigenic potency as well as for anticomplementary activity. Usually the latter activity was low, while the antigenic potency was high; when a "box titration" against homologous monkey antiserum was carried out, the antigen diluted to 1:8 still reacted with the antiserum at a dilution of 1:256. Four antigenic units of antigen were used for screening the sera, one unit being defined as the highest dilution of antigen (contained in 0.1 ml.) that reacted with dilute (at least 1:32) homologous monkey antiserum. Human sera were run at dilutions of 1:4 and 1:16.

RESULTS

I. Prevalence of Neutralizing and Complement-Fixing Antibodies.

The age-specific spring and fall prevalence of antibodies in paired sera is shown in Figure 2. The neutralizing antibody patterns are similar to those previously reported. In the lower socioeconomic group (District 1) living in an insanitary environment, there was a rapid increase in the earlier years of life, after which the high level of antibodies was maintained. In the upper middle class group (District 4), neutralizing antibodies rose more slowly, to attain a prevalence of 50% only after age 15. High levels were reached only in young adulthood.

It appears that Type 2 virus was producing infections in District 4, but there is no evidence of its presence in District 1 during the summer of 1951. This is in contrast to the situation which must have been present in District 1 in the immediately preceding years, for by the spring of 1951 there were 15 positive of 23 children of ages one through three years.

The prevalence of C-F antibody provides an additional measure of infection. This antibody began to rise at an early age in both districts, but reached a prevalence of 50% in District 1 earlier than in District 4. In the fall in District 1, at ages under 20 years, the prevalence ratio of C-F antibody in those groups in which neutralizing antibody was not demonstrated was 16% at 1:4 and 2% at 1:16 serum dilution, as compared with a prevalence in those showing neutralizing antibody of 51% at 1:4 and 25% at 1:16. In District 4, 10% of sera not showing neutralizing antibody had C-F antibody at 1:4 and 3% at 1:16 serum dilution; 67% of sera with neutralizing antibody showed C-F antibody at 1:4 level and 20% at a titer of 1:16.

With regard to the average persistence in these populations of C-F antibody at the level measured, the loss over the observation period of six months was very small. No reversions were observed in District 1. In

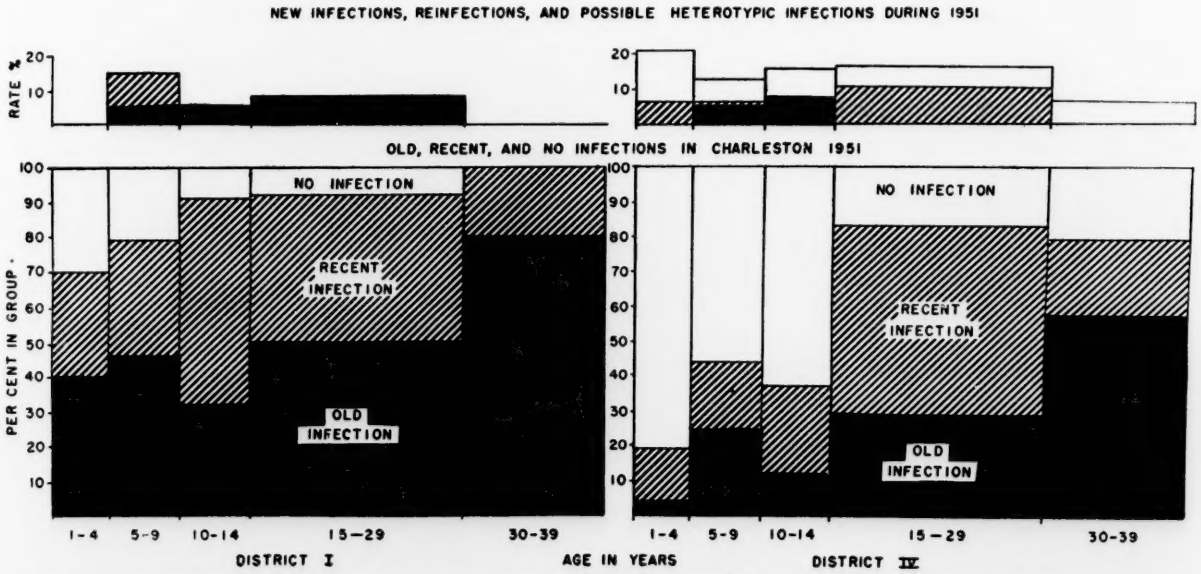

FIG. 3. The lower charts show the age distribution of persons with *old, recent,* or *no* infections in the two study districts prior to 1951. The upper charts show infections identified as occurring during the summer of 1951; and within each age group, persons are further classified according to whether they arose from the groups showing *old, recent,* or *no* infections.

Criteria used for grouping:

	Antibody status			
	Spring		*Fall*	
	Neut.	*C-F*	*Neut.*	*C-F*
No evidence of infection:	—	—		
"Old infections:	+	—		
"Recent" infections				
Group A—Probably homotypic:	+	+		
Group B—Probably heterotypic:	—	+		
"New" infections, 1951				
Group A:	—	—	+	+
Group B:	—	—	+	—

	Antibody status			
	Spring		*Fall*	
	Neut.	*C-F*	*Neut.*	*C-F*
"Reinfections" or heterotypic infections, 1951				
Group A:	+	—	+	+
Group B:	+	+	+	++
Group C:	—	—	—	+
Group D:	—	+	—	++

District 4, two of 19 sera positive at 1 : 4 serum dilution in the spring were negative in the fall, and one serum of 13 positive at 1 :16 serum dilution in the spring fell to 1 : 4 in the fall. Whether the persistence is a matter of duration after single infection or is due to reinfection with homo- or heterotypic strains is not evident here.

Figure 3 is a schematic representation of the information from the combined tests. As has been noted, the type specificity of individual C-F reactions cannot readily be determined, but the general pattern of old, recent, and new infections as defined in the legend of the figure can be observed. "Old" infections—i.e., persons possessing neutralizing but lacking C-F antibodies—were more prevalent in District 1 at all ages under 40 years. The picture is not like that shown by neutralizing antibody alone in that prevalence is much lower in both districts, and the difference between districts seems to be of greater magnitude. There was little or no increase with age between 5 and 30 years. The age pattern of prevalence of "recent" infections prior to 1951—i.e., persons with C-F antibodies in the spring of 1951—adds more information which is not provided by the neutralizing antibody pattern alone. Infections in this category were more numerous in District 1 at ages 1 through 14, but no difference between districts was noted at ages 15-39. The prevalence of "recent" infections rose early in District 1 and not sharply in District 4 until after the age of 15 years.

"New" infection rates differed in the two districts. No new infections—i.e., persons lacking neutralizing or C-F antibodies in the spring who developed them by the fall—were evident in 1951 in District 1 among the 17 persons with no evidence of previous infection, while significant numbers occurred in District 4 as follows: 6/26 (23%) at ages 1-4 years, 4/35 (11%) at ages 5-9, 3/24 (12%) at ages 12-14, and 2/6 persons at ages 15-39 years. Five of the new infections at ages 1-14 years were indicated by development of C-F antibody only. One of these, age 12, was in a household in which two siblings of ages 2 and 16 years developed both neutralizing and C-F antibodies and one sibling, age 13, with neutralizing antibody in the spring developed C-F antibody during the summer.

"Re-infections"—i.e., persons possessing neutralizing antibodies in the spring who developed or showed an increase in C-F antibodies in the fall—were similar in the two districts. As there appears to have been an absence of "new" infections with Type 2 virus in District 1, this suggests that "re-infection" is probably at least partially heterotypic infection. Three of six children in District 4 who developed C-F antibodies with neutralizing antibodies demonstrated in the spring and fall were in households with siblings

who developed both neutralizing and C-F antibodies during the summer of 1951.

II. Association between Neutralizing Antibody and Certain Variables in Socioeconomic Status and Environment.

District 1. The age-specific prevalence of Type 2 neutralizing antibody in the fall of 1951 is shown for both districts in Figure 2. There were 54 families in District 1 from whom sera of children between the ages of one

TABLE 1. COMPARISON OF ENVIRONMENTAL FACTORS OF DISTRICT 1 HOUSEHOLDS WITH CHILDREN OF AGES 1-8 TESTED FOR PRESENCE OF NEUTRALIZING ANTIBODY, IN HOUSEHOLDS WITH NEGATIVE SERUM, AND IN HOUSEHOLDS WITH ONE OR MORE POSITIVE SERA

					Economic class		*Living quarters*			
Households	*No.*	*Av. family size*	*Persons per room*	*Av. no. persons under 15 years*	*Low-middle*	*Low*	*Clean*	*Fairly clean*	*Not clean*	*No running water*
With negative sera*	29	4.9	1.2	2.2	9	20	6	18	5	1
With positive sera†	25	7.1	2.1	4.4	3	22	2	4	19	6
P‡			$<.001$	$<.001$					$<.001$	.05

* Households with no child showing neutralizing antibody.
† Households in which one or more children had neutralizing antibody.
‡ Probability of chance difference.

and eight years were examined. Neutralizing antibody was detected in the serum of one or more children in 25 of these families, while in 29 households none was demonstrated. Table 1 shows some of the socioeconomic and environmental characteristics of these families. It is evident that the children with neutralizing antibody tended to come in greater proportion from families with larger numbers of children, and from more crowded and poorer living quarters. The interviewers were instructed, in rating of cleanliness of living quarters, to omit considerations of attractiveness or general tidiness, and to record only the evidence of regular cleaning or the accumulation of dirt over a period of time. The rating of "fairly clean" indicated something less than a spotless condition, but those premises did show evidence of regular use of soap and water, and will be included in the "clean" category in this discussion. No comparison of outside premises has been included because no differences were observed. All blocks were rated

poor. The number of children in families and cleanliness of living quarters were closely associated, with cleanliness decreasing as the number of children increased. The average number of children under 15 years of age was 2.0 in clean households, 2.5 in fairly clean, and 4.0 in households rated not clean.

The prevalence of neutralizing and C-F antibodies in children one through eight years of age in households rated clean and not clean is shown in Table 2. In clean households with 1-4 children, only three of 33 children

TABLE 2. RELATIONSHIP BETWEEN CLEANLINESS OF LIVING QUARTERS AND PREVALENCE OF ANTIBODIES IN CHILDREN OF AGES 1-8 YEARS LIVING IN DISTRICT 1

Household sanitary rating	*Number under 15 years*	*Neutralizing antibody*		
		Number of tests	*Number positive*	*Expected* number positive*
Clean	1-2	24	1	9.6
	3-4	9	2	6.0
	5-6	9	7	6.8
	Total	42	10	22.4
Not clean	1-2	5	2	0.2
	3-4	24	16	5.3
	5-8	25	19	19.5
	Total	54	37	25.0

* Number of positive neutralization tests expected in each group, at ages 1-8, at observed ratio in families of the same size in the other group.

aged one through eight years were positive, as compared with 18 of 29 in families rated as not clean. The difference was more marked at ages 5-8 than at 1-4 years. In families with five or more children, no difference related to cleanliness was noted. Preschool children in households rated "not clean" showed a higher antibody prevalence (14/21), when older children were also present, than did those in families with preschool children only (1/6). This difference cannot be shown to be independent of family size in the numbers observed. Prevalence of neutralizing antibody in preschool children in clean households was the same in families with older children (1/9) and with no older children (2/15).

District 4. Children tested between the ages of one and 14 years came from 112 households in 42 blocks. Socioeconomic status was recorded as upper middle class for 29 families and middle class for 83. All living quarters and individual premises were clean. The general cleanliness rating of

TABLE 3. PREVALENCE OF ANTIBODIES IN CHILDREN 1-14 YEARS OF AGE IN MIDDLE AND UPPER MIDDLE CLASS HOUSEHOLDS IN BLOCKS WITH GOOD AND FAIR CLEANLINESS—DISTRICT 4

No. under age 15 in Household	Age	Neutralizing antibody						C-F antibody 1:4 serum dilution					
		Fair blocks		Good blocks				Fair blocks		Good blocks			
				Middle class		Upper middle				Middle class		Upper middle	
		Tests	Positive	Tests	Positive	Tests	Positive	Tests	Positive	Tests	Positive	Tests	Positive
1-2	1-4	10	4	28	5	4	0	9	2	25	4	4	1
	5-9	13	6	21	5	7	2	13	5	18	4	4	0
	10-14	6	3	10	3	9	1	6	1	10	3	9	2
	1-14	29	13	59	13	20	3	28	8	53	11	17	3
3 and over	1-4	1	1	8	1	10	7	1	1	8	3	8	6
	5-9	2	1	18	7	19	12	1	1	13	0	18	8
	10-14	0		10	6	5	2			9	7	4	2
	1-14	3	2	36	14	34	21	2	2	30	10	30	16

34 blocks was good, while eight blocks were rated as fair. In the latter group there were no major violations of environmental hygiene standards. The lower rating was given because of deviations in garbage handling which provided fly attractant material in alleys, or because of a few premises with some deterioration of structures, and with poorly kept yards.

Neutralizing antibody was shown to be present in one or more children 1-14 years old in 40 households and was not found in children of the same ages in 72 households. Differences between the two groups in socioeconomic and environmental characteristics were not remarkable. The average number of children was slightly but not significantly larger in families in which neutralizing antibody was found. The observed prevalence of neutralizing and C-F antibodies in children tested in "fair" and "good" blocks is shown in Table 3. Children in families with one or two children under 15 years of age in "fair" blocks showed higher levels of neutralizing antibody at all ages under 15 than did those in "good" blocks. In families with three or more children, those of ages 1-9 years from the upper middle class group had a higher level of neutralizing antibody than those from families rated as middle class. This was most marked at ages 1-4 years where seven of ten upper class children tested showed neutralizing antibody as compared with one child of eight tested in the middle class group. In both middle and upper middle class families, prevalence of neutralizing antibody was higher in the larger families, but this difference was marked only in the upper middle class group. In the middle class children, the difference was apparent at ages 5-14 only. There was no evidence of an increase in prevalence of neutralizing antibodies at ages 10-14 years in middle or upper middle class families. Six of the negative 10-14-year-old children in the two groups were in households with younger children who had neutralizing antibodies.

III. The Relationship between the Number of Children in Families and the Prevalence of Type 2 Poliomyelitis Antibody.

It has been evident from the data presented from each district that prevalence of neutralizing antibody was related to the number of children in families. This association which has been noted in earlier studies[4,5] is shown clearly in Table 4. In each age group in each district the prevalence ratios increased as the number of children under 15 years of age in families increased, except in children aged 10-14 years in the larger families in District 1. Here 25 of 27 children tested in the spring and 21 of 24 tested in the fall were positive.

It was pointed out earlier that differences in the age-specific prevalence of antibodies between the two districts were consistent with previous

observations of a high prevalence of antibodies from lower socioeconomic strata and slower accumulation in children of middle and upper middle class families. Such differences have been interpreted as being related to living conditions and sanitary environment, and this is indicated further by differences within districts associated with environmental factors. In the spring, the observed prevalence of neutralizing antibody at all age groups under 15 was distinctly higher in substandard District 1. After a summer during which new infections were occurring in District 4 and none were evident in District 1, the differences between the districts at older ages were maintained, but prevalence of neutralizing antibody in children under five was similar in the two districts. It is evident from the data presented in Table 4 that no differences were observed between the districts in children under ten years of age in families with one and two children under 15, and if the observed ratios in each district are applied to the average or "standard" distribution of children by family size, adjusted ratios indicate that certain differences between districts could be accounted for by the presence of a higher proportion of larger families in District 1.

IV. The Age-specific Prevalence of Type 2 Poliomyelitis Antibodies in the Fall of 1951 Considered in Relation to the Incidence of Clinical Poliomyelitis in the Summer of 1952.

From the data presented in the preceding sections it appeared that there were differences in the age-specific patterns of antibody accumulation which seemed to be associated with socioeconomic and environmental factors. Individuals in families classified as low in socioeconomic status appeared in general to accumulate neutralizing antibody at a more rapid rate than those in middle class groups. It was evident that a high level of neutralizing antibody was not necessarily associated with a decrease in recent infections as indicated by the C-F reaction, but no direct evidence in regard to the possible effect on clinical disease could be obtained here because of the small numbers in the population groups under study. For such evidence, clinical poliomyelitis in the entire city during 1951 and 1952 was studied.

Although changes in antibody prevalence indicated that a significant number of infections were occurring, in 1951 only four cases were reported, two paralytic and two non-paralytic, with one onset each in April, July, September, and December. In 1952, however, 27 cases were clinically diagnosed in a metropolitan population district of about 125,000. At the time of investigation of each case household, the epidemiologist recorded environmental and socioeconomic observations of the same kind as were made in the study areas. It is not possible to say that the groups classified

TABLE 4. THE RELATIONSHIP BETWEEN THE NUMBER OF CHILDREN UNDER 15 YEARS OF AGE IN HOUSEHOLDS AND THE PREVALENCE OF POLIOMYELITIS TYPE 2 NEUTRALIZING ANTIBODIES IN CHILDREN OF AGES 1-14

			Ages 1-4		*Ages 5-9*		*Ages 10-14*		*Total ages 1-9*			
											Adjusted	
	District	*Number in household under 15*	*Tests*	*Positive*	*Tests*	*Positive*	*Tests*	*Positive*	*Observed Tests*	*Positive*	*Standard pop.†*	*Expected pos.‡*
Spring	1	1	1	0	3	1	3	3	4	1	10	2.5
		2	8	3	6	2	3	3	14	5	30.5	11.0
		3	9	5	9	7	3	3	18	12	28	18.8
		4–5	5	4	20	17	10	9	25	21	17	14.3
		6–8	9	8	16	14	8	7	25	22	12.5	11.0
	4	1	6	0	10	1	4	1	16	1	10	.6
		2	18	3	29	14	21	5	47	17	30.5	11.0
		3	12	3	26	10	9	3	38	13	28	9.5
		4–5	1	1	8	8	5	4	9	9	29.5	29.5
	1	1–2	9	3	9	3	6	6	18	6	40.5	13.5
	4	1–2	24	3	39	15	25	6	63	18	40.5	11.6
		P*		.35		1.0		$< .01$		.55		
	1	1–8	32	20	54	41	27	25	86	61	98	57.6
	4	1–5	37	7	73	33	39	13	110	40	98	50.6
		P*		$< .001$		$< .001$		$< .001$		$< .001$		

Fall	1	1	6	0	4	2	4	4	10	2	17	3.4
		2	14	2	9	2	3	3	23	4	41	7.0
		3	9	3	6	4	3	3	15	7	31	14.6
		4–5	10	6	20	16	7	5	30	22	20.5	15.0
		6–8	11	7	17	15	7	6	28	22	14	11.0
	4	1	14	2	10	1	5	1	24	3	17	2.0
		2	28	7	31	12	20	6	59	19	41	13.1
		3	17	7	30	13	10	4	47	20	31	13 3
		4–5	2	2	9	7	5	4	11	9	34.5	28.3
	1	1–2	20	2	13	4	7	7	33	6	58	10.4
	4	1–2	42	9	41	13	25	7	83	22	58	15.1
		P*		.45		1.0		.003		.34		
	1	1–8	50	18	56	39	24	21	106	57	123.5	51
	4	1–5	61	18	80	33	40	15	141	51	123.5	56.7
		P*		.42		$< .005$		$< .001$		.005		

* Probability of chance occurrence of difference.

† Standard population is the average of the two observed distributions.

‡ Expected positives at observed ratio if distributed in families as in the standard population.

District 1. Substandard environment, low and low middle class.

District 4. Standard environmental hygiene, middle and upper middle class.

in the study areas are a truly representative sample of these classes in the entire city. However, the classification of case households was comparable to the rating of families in the study, and the age distributions of children in households were similar for each group.

The distribution of recognized cases of poliomyelitis according to the above grouping is shown in Figure 4 together with the antibody patterns observed in the families with similar classifications in the study districts. Only three of the cases reported were from families classified as of low socioeconomic status. Ages were five months, 5 and 12 years. The infant had severe paralysis, but the other cases were non-paralytic. The remainder of the cases, 17 paralytic and 7 non-paralytic, came from middle class families. Paralytic cases appeared to be distributed more or less uniformly from 3-14 years of age. Since we do not know the population of the several socioeconomic groups in the city, no accurate comparison of attack rates can be made. A crude estimate based on the original survey indicated that roughly 25% of families in Charleston were in the low classification. If the average number of children under 15 years of age is larger in families in this group, as indicated by the study populations, the population under 15 in the low socioeconomic level should be well over 25% of the total in the city of these ages. If this is so, these children were not providing clinical cases at the same rate as those of middle class status at ages beyond infancy.

DISCUSSION

Antibody patterns of the two districts. A comparison of the Charleston results with an earlier survey by the same methods carried out in Cairo, Egypt, is revealing (see Fig. 5). Neutralizing and C-F antibodies are acquired at a very early age in this subtropical, crowded city.[3,7] In contrast to neutralizing antibodies which were retained for life, C-F antibodies were confined to childhood. The development of immunity to poliomyelitis in Charleston, in contrast to Cairo, is a prolonged process, particularly in the middle and upper middle class populations of District 4. Antibody development in the lower socioeconomic group (District 1) proceeded at a rate intermediate between that observed in Cairo and in District 4.

Adults 40 years of age and older, even in District 4, had high levels of neutralizing antibody. The experience of this group with poliomyelitis virus in earlier years may have been different from that occurring more recently in this kind of population. It is possible that in their childhood they may have developed and maintained neutralizing antibodies under circumstances similar to those recently observed in Cairo.

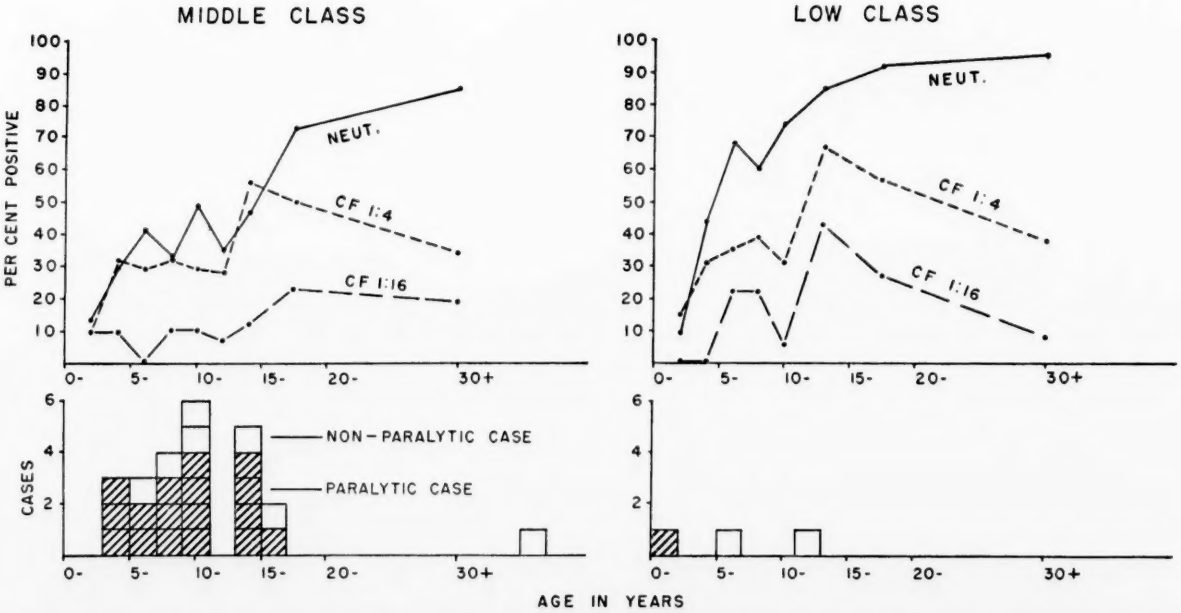

FIG. 4. Prevalence of Type 2 antibodies in children in low and middle class households in the fall of 1951 and the age distributions of poliomyelitis cases in these socioeconomic groups the following summer. Between 11 and 62 healthy persons were present in each age group which contributed sera.

The presence of C-F antibody in sera not showing neutralizing antibody may be due to Type 2 infections with neutralizing antibody titers below the screening level used, or more likely, to infection with another type of poliomyelitis virus. These sera do not change the pattern of age-specific prevalence of C-F antibody as an indication of prevalence of recent poliomyelitis infection. The question of specificity arises also in the interpretation of a rise in titer of Type 2 C-F antibody in the presence of neutralizing antibody. Again, it is not possible from the evidence presented to say whether this indicates a reinfection with Type 2 virus or infection with another type.

The prevalence of C-F antibodies at all ages measured is evidence that infections are not rare in District 4. The average persistence of this antibody, at the levels measured, after a single subclinical infection is not precisely known. The Cairo observations[8] indicated that the C-F antibody did not persist for long periods. If the presence of C-F antibodies means infection within a year or two, incidence of infection in both districts in Charleston must have been rather high at all ages for the year or two immediately preceding the collection of these sera. "Old" infections had not steadily accumulated in either district until after age 30. Young adults who contributed sera positive for C-F antibodies had been subjected to household contact with infection. In spite of this background of exposure, they had not reached a level of immunity high enough to prevent infections. It is possible that failure to maintain high levels of immunity may be a matter of size of dose as well as lack of repeated stimuli.

This interpretation of the data may have a bearing on the requirements for success of a vaccination program. If vaccine is needed which will produce titers comparable to those developed by natural infections, it is likely that frequent repeated stimulation will be necessary to maintain a high level of immunity. This kind of stimulation cannot be expected to occur naturally to an adequate extent in populations like those in District 4.

Association of antibodies and environment. In District 1, in spite of the generally poor environment, antibodies in children of ages 1-8 were not distributed uniformly or at random, but tended to reach a higher prevalence in families with large numbers of children in crowded, unclean living quarters. It appears that adequate use of soap and water, or some closely related variable, can reduce the spread of poliomyelitis virus, at least in younger children, even in the presence of abundant sources of virus in the outside environment. Whether infection was actually prevented is questionable, but apparently it did not occur often enough, or possibly in sufficient dose, to build up and maintain a high level of neutralizing antibody in the younger

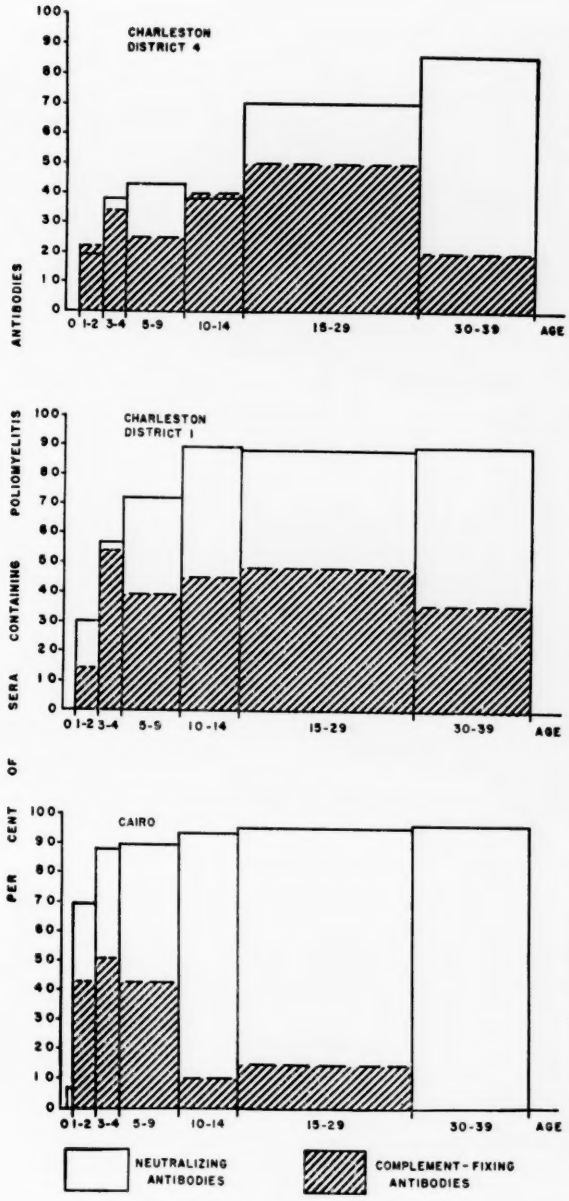

FIG. 5. Age-specific prevalence of Type 2 neutralizing and C-F antibodies in Cairo, Egypt, and Districts 1 and 4 in Charleston. Data for Cairo taken from references 3 and 7.

children. In District 4, the prevalence of neutralizing antibody in smaller families appeared to decrease as socioeconomic status and block hygiene improved, but in larger families this was not true. Children under 10 years of age in *large* upper middle class families showed levels of neutralizing antibody comparable to those observed in the lowest group in District 1.

The strong effect of household aggregation of children on the prevalence of neutralizing antibody at all socioeconomic and environmental hygiene levels is evident in this study. Spread within households seems to be a potent factor in building up and maintaining immunity during the earlier years of life. However, among older children in District 1 the high antibody level appears to have been reached and maintained independent of household contact, and in District 4 the effect of household aggregation is not as marked after the age of ten.

The data suggest that infection with poliomyelitis virus in younger children is most likely to be acquired from household contact with older children, and that this kind of contact favors maintenance of antibody levels. In the insanitary district, older children apparently had acquired and maintained immunity with or without household contact with other children. In the cleaner environment of District 4, even in large families, the children over nine years of age did not appear to develop or perhaps to maintain neutralizing antibody at high level. At some time during the adolescent period there appeared to be a high infection rate as indicated by the presence of C-F antibodies. This seemed to occur at a somewhat earlier age in District 1, and in the presence of neutralizing antibody. In District 4, C-F antibody patterns suggested that reinfections were still taking place among the parents of the children. Whether this kind of pattern would be seen in adults between 20-40 years of age who are not in contact with children remains to be investigated. The present serological evidence lends support to the view that poliomyelitis virus spreads much more readily within families than from family to family.

Antibody and clinical disease. Present concepts suggest that in any large population group, not isolated over a period of a few years, all types of poliomyelitis virus should be present and produce infections and antibodies in a similar fashion. Thus the patterns observed for any one type should indicate the level of immunity to all.* Recent studies[7] have shown that, in

* Another interpretation is that Type 2 infection may protect against Type 1 and Type 3 infections. In support of this view, recent data (Fed. Proc., Balt., 1955, *14*, 472) have been obtained which have revealed an antigenic overlap among the poliomyelitis viruses. Thus, Type 1 and 2 viruses are found to have antigens in common. Crossing was also commonly observed between Type 2 and 3 strains, but not between Types 1 and 3.

certain areas of the globe, the age distribution of clinical cases is in proportion to that of Type 2 subclinical infections, and here within a single city the same principle seems to hold. In Charleston, in the lowest socioeconomic group living under the environment most deficient in sanitation, rapi development of high levels of neutralizing antibody was observed early in life, with "recent" infections continuing to occur later in life. One might expect clinical cases to appear early in life, and possibly again in decreased proportion and lessened severity at periods of "recent" infections. The more severe clinical manifestations should be rare except early in life, and possibly again in decreased proportion and lessened severity at periods of "recent" infections. The more severe clinical manifestations should be rare except early in life. In the smaller and cleaner families in the insanitary areas and in families in the better hygienic environment, neutralizing antibody rises more slowly. Cases might be expected to occur over a longer period of life. Actually, the 1952 cases in Charleston were distributed according to age in the two population groups as expected from the age-specific prevalence of antibody in the fall of 1951. The concept of gradually acquired immunity which is maintained by repeated stimuli might well account for the patterns which have been observed.

SUMMARY

The 1951 spring and fall prevalence of neutralizing and complement-fixing antibodies to poliomyelitis virus in two population groups of Charleston, West Virginia, differing in socioeconomic status and sanitary environment, has been described.

Age-specific patterns of neutralizing antibody prevalence in the two districts conform to those previously described in that the population of low socioeconomic status in an insanitary environment developed antibodies at a rapid rate and maintained high levels through the older ages. In the upper middle class group, antibodies rose more slowly, reaching high levels only in young adulthood. In addition to the way of life determined by socioeconomic status, the aggregation of children in households appeared to be a potent factor in determining the prevalence of neutralizing antibody within families.

Complement-fixing antibody was at peak prevalence at ages between 10 and 20 years in both populations, and was still present in a substantial proportion of adults 20-40 years of age. This is in contrast to findings in subtropical, crowded, insanitary areas.

The age selection of clinical cases of poliomyelitis occurring in the summer of 1952 was related to the age-specific prevalence of Type 2 polio-

myelitis antibodies in the fall of 1951, both in the low and in the middle class populations.

The data obtained support the hypothesis that in this urban area immunity is developed slowly and even in a substandard environment probably is well maintained only with repeated stimuli. In addition to environmental sanitation the number of children in families is important in influencing the development and maintenance of immunity.

ACKNOWLEDGMENTS

In a project of this type a large number of people must necessarily participate. Grateful acknowledgment is made for the interest and co-operation of the West Virginia State Department of Health and the Charleston-Kanawha County Health Department. Particular thanks are extended to Dr. Ira L. Myers and the staff of the Charleston Project for their aid in the field. We also wish to acknowledge the assistance of Dr. Natan Goldblum who participated in certain phases of the laboratory work.

REFERENCES

1 Casals, J., Olitsky, P. K., and Anslow, R. O.: A specific complement fixation test for infection with poliomyelitis virus. J. Exp. M., 1951, *94*, 123-137.

2 Casals, J., Olitsky, P. K., and Sabin, A. B.: Development, persistence and significance of Type 2 poliomyelitis complement fixing antibody in man. J. Exp. M., 1952, *96*, 35-53.

3 Goldblum, N. and Melnick, J. L.: Complement fixing antibodies to Type 2 (Lansing) poliomyelitis virus in a normal population of a subtropical area. J. Exp. M., 1952, *96*, 175-185.

4 Hammon, W. McD., Sather, G. E., and Hollinger, N.: Preliminary report of epidemiological studies on poliomyelitis and streptococcal infections. Lansing neutralizing antibody and anti-streptolysin "o" surveys of California cities, Texas, North Carolina, Mexico, Pacific Islands, and Japan. Am. J. Pub. Health, 1950, *40*, 293-306.

5 Melnick, J. L. and Ledinko, N.: Social serology: Antibody levels in a normal young population during an epidemic of poliomyelitis. Am. J. Hyg., 1951, *54*, 354-382.

6 Melnick, J. L. and Ledinko, N.: Development of neutralizing antibodies against the three types of poliomyelitis virus during an epidemic period. The ratio of inapparent infection to clinical poliomyelitis. Am. J. Hyg., 1953, *58*, 207-222.

7 Paul, J. R., Melnick, J. L., and Riordan, J. T.: Comparative neutralizing antibody patterns to Lansing (Type 2) poliomyelitis virus in different populations. Am. J. Hyg., 1952, *56*, 232-251.

8 Svedmyr, A., Enders, J. F., and Holloway, A.: Complement fixation with three types of poliomyelitis viruses propagated in tissue culture. Am. J. Hyg., 1953, *57*, 60-70.

9 Turner, T. B., Hollander, D. H., Buckley, S., Kokko, U. P., and Winsor, C. P.: Age incidence and seasonal development of neutralizing antibodies to Lansing poliomyelitis virus. Am. J. Hyg., 1950, *52*, 323-347.

10 Walton, M. and Melnick, J. L.: Coxsackie virus antibody and incidence of minor illness during the summer. Pub. Health Reports, 1953, *68*, 1167-1178.

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THE EFFECTS OF PROPYLTHIOURACIL AND THYROTROPIC HORMONE ON THE UPTAKE OF RADIOACTIVE THALLIUM BY THE RAT THYROID

In studies of the organ distribution of radioactive thallium (Tl^{204}) in the rat, Thyresson[8] and Barclay, *et al.*[1] found that on a gram-per-tissue basis this isotope was taken up by the thyroid in large amounts. Twenty-four hours after acute poisoning with a single large dose of Tl^{204}, the kidney took up the greatest amount of isotope, followed by the colon and the thyroid in that order.[8] It was with the object of determining whether propylthiouracil and thyrotropic hormone could alter the thyroid uptake of Tl^{204} in a manner similar to that of I^{131}, that the following experiments were performed.

METHOD

General. Albino male rats of the Sprague-Dawley strain, with an average body weight of 250 grams, were used. The animals had free access to water and were fed Purina laboratory dog chow *ad libitum.* Approximately 5 microcuries of radioactive thallium‡ ($Tl^{204}(NO_3)_2$, specific activity = 239 millicuries/gram) dissolved in 0.5 cc. of isotonic saline were injected intraperitoneally into each rat. The animals were sacrificed by anesthetizing with ether and withdrawing blood from the exposed heart with a heparinized syringe. One-half cc. portions of whole blood and plasma were placed into cupped nickel-plated steel planchets (Tracerlab) and air dried. The wet weights of the thyroid, pituitary, and adrenals were determined on a Roller-Smith precision balance and the organs were dried in an oven at 100° C. for 24 hours, re-weighed, and placed into cupped planchets. A Geiger-Mueller counter (Tracerlab) with an end window thickness less than 2 mg./cm.^{2} was used for measuring the radioactivity of each sample.

Propylthiouracil. Forty rats were divided into two groups of 20 animals. The experimental group received powdered Purina laboratory dog chow to which had been added propylthiouracil so as to make a 0.1 per cent concentration of the drug. Twelve days after the start of this dietary regimen all animals were injected with Tl^{204} and sacrificed at intervals of ½ to 96 hours thereafter.

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‡ Obtained from the U.S. Atomic Energy Commission.

Received for publication March 22, 1955.

*Thyrotropic hormone.** Fifty-two rats were injected with Tl^{204} and 12 hours later 26 of these animals received a subcutaneous injection of 5 mg. thyrotropic hormone (TSH). Those rats sacrificed 48 hours after receiving the initial dose of TSH received a second injection of 5 mg. TSH 24 hours after the first. Those rats sacrificed 72 hours after the initial TSH injection received a second 5 mg. TSH injection 24 hours after the first and a third dose of 3 mg. 24 hours later.

TABLE 1. THE UPTAKE OF RADIOACTIVE THALLIUM (Tl^{204}) BY THE THYROID OF RATS TREATED WITH PROPYLTHIOURACIL

Hours after Tl^{204} injection	*Per cent dose x 10^{-3}*		*Per cent dose/mg. wet weight x 10^{-4}*		*Per cent dose/mg. dry weight x 10^{-3}*	
	Propylthiouracil	*Control*	*Propylthiouracil*	*Control*	*Propylthiouracil*	*Control*
½	10.2 ± 3.1** P > .05 < .1 (3)†	2.0 ± 0.3 (2)	2.7 ± .03	2.4 ± 0.65	1.5 ± 0.2	1.1 ± 0.3
1	15.9 ± 0.3 P << .001 (2)	2.8 ± 0.6 (3)	4.4 ± 0.5 P > .05 < .1	2.5 ± 0.5	2.5 ± 0.3 P < .05	1.2 ± 0.3
3	11.2 ± 2.6 P < .05 (3)	3.1 ± 0.4 (3)	3.2 ± 0.5	2.6 ± 0.3	1.9 ± 0.3 P < .1 > .05	1.2 ± 0.15
8	15.7 ± 2.8 P < .02 (2)	3.2 ± 0.3 (3)	4.9 ± 0.07 P < .02	3.6 ± 0.3	3.1 ± 0 P < .01	1.6 ± 0.2
24	13.4 ± 0.7 P << .001 (2)	3.2 ± 0.4 (3)	3.5 ± 0.5	2.9 ± 0.2	2.0 ± 0.2 P < .1 > .05	1.3 ± 0.2
48	10.4 ± 0.2 P << .001 (2)	2.0 ± 0.15 (3)	2.5 ± 0.1 P < .1 > .05	2.0 ± 0.2	1.5 ± 0 P << .001	0.8 ± 0.04
96	16.0 ± 1.3 P << .001 (3)	1.7 ± 0.4 (3)	3.1 ± 0.09 P < .01	1.6 ± 0.3	1.5 ± 0.15 P < .01	0.6 ± 0.1

** Mean ± standard error.
† Number of animals in each group.
"P" not recorded unless it is .05 or less.

In the second TSH experiment, 16 rats received two daily subcutaneous injections of 4.5 mg. TSH for two days, or a total of 18 mg. Immediately following the fourth

* We wish to thank Dr. Sanford L. Steelman of the Armour Laboratories for a generous supply of thyrotropic hormone (Lot number P589-80, 0.9 USP units/mg.).

injection, the experimental animals and their untreated controls were injected with Tl^{204} and sacrificed at intervals of 12 to 72 hours thereafter.

RESULTS AND DISCUSSION

Propylthiouracil. The uptake of Tl^{204} by the thyroids of the PTU-treated rats was significantly greater than that of the controls (Table 1). When

TABLE 2. THE LEVEL OF RADIOACTIVE THALLIUM (Tl^{204}) IN THE BLOOD AND PLASMA OF RATS TREATED WITH PROPYLTHIOURACIL

Hours after Tl^{204} injection	*Per cent dose x 10^{-1}*		*Per cent dose x 10^{-1}*	
	Blood‡		*Plasma‡*	
	Propylthiouracil	*Control*	*Propylthiouracil*	*Control*
½	4.93 ± 0.92* (3)†	4.10 ± 0.70 (2)	1.76 ± 0.43	1.31 ± 0.35
1	5.44 ± 0.64 (2)	3.80 ± 0.86 (3)	1.69 ± 0.27	1.33 ± 0.31
3	3.26 ± 0.71 (3)	3.28 ± 0.65 (3)	1.03 ± 0.20	0.99 ± 0.20
8	2.85 ± 0.02 (2)	2.60 ± 0.13 (3)	0.92 ± 0.05	0.85 ± 0.08
24	1.90 ± 0.26 (2)	2.11 ± 0.17 (3)	0.60 ± 0.10	0.64 ± 0.03
48	1.51 ± 0.09 (2)	1.32 ± 0.03 (3)	0.50 ± 0.01	0.42 ± 0.02
96	1.00 ± 0.007 (3)	0.98 ± 0.09 (3)	0.34 ± 0.004	0.30 ± 0.02

* Mean ± standard error.
† Number of animals in each group.
"P" not recorded unless it is .05 or less.
‡ Values of 4.95 cc. and 2.63 cc. obtained by Sharpe, *et al.*[8] were used for calculating the total blood and plasma volumes, respectively.

the Tl^{204} uptake by the gland was calculated on a weight basis, the PTU-treated animals still showed a greater thyroid Tl^{204} content, even though statistically significant differences could not be demonstrated consistently. Up to three hours following injection of the isotope, the greater Tl^{204} uptake of the PTU-treated rats was small and of doubtful significance. However, at eight hours and thereafter, the magnitude of the increased

uptake of Tl^{204} by the thyroids of the PTU-treated rats became greater and reached approximately twice the control values at 48 and 96 hours.

The increased Tl^{204} uptake by the thyroids of PTU-treated rats is analogous to the results obtained by Durbin, *et al.*[2] and by Shellabarger and

TABLE 3. THE UPTAKE OF RADIOACTIVE THALLIUM (Tl^{204}) BY THE PITUITARY OF RATS TREATED WITH PROPYLTHIOURACIL

Hours after Tl^{204} injection	*Per cent dose x 10^{-3}*		*Per cent dose/mg. wet weight x 10^{-4}*		*Per cent dose/mg. dry weight x 10^{-5}*	
	Propylthiouracil	*Control*	*Propylthiouracil*	*Control*	*Propylthiouracil*	*Control*
½	2.1 ± 0.3* P < .01 (3)†	1.4 ± 0.1 (2)	2.4 ± 0.4	1.6 ± 0.4	1.4 ± 0.3	0.8 ± 0.2
1	2.1 ± 0.6 (2)	1.4 ± 0.2 (3)	3.3 ± 0.6	1.7 ± 0.4	1.6 ± 0.5	0.8 ± 0.2
3	2.1 ± 0.2 (3)	1.8 ± 0.2 (3)	2.6 ± 0.3	2.2 ± 0.3	1.3 ± 0.1	1.2 ± 0.2
8	1.8 ± 0 P < .05 (2)	1.5 ± 0.09 (3)	2.4 ± 0.07 P < .01	1.8 ± 0.04	1.3 ± 0.07 P < .05	1.0 ± 0.07
24	3.0 ± 0.1 (2)	3.0 ± 0.3 (3)	3.5 ± 0.3	3.8 ± 0.3	1.8 ± 0.07	1.9 ± 0.2
48	1.5 ± 0.2 P < .02 (2)	0.7 ± 0.06 (3)	1.4 ± 0.2 P < .05	0.8 ± 0.04	0.8 ± 0 P << .001	0.4 ± 0
96	4.9 ± 0.07 P < .01 (3)	3.4 ± 0.3 (3)	5.4 ± 0.4 P < .05	4.1 ± 0.3	3.0 ± 0.4 P < .05	1.9 ± 0.1

* Mean ± standard error.
† Number of animals in each group.
"P" not recorded unless it is .05 or less.

TABLE 4. ADRENAL WEIGHTS OF RATS TREATED WITH PROPYLTHIOURACIL

	Wet Weight mg.	*Dry Weight mg.*
Propylthiouracil (20)*	27.62 ± 0.18† P << .001	9.43 ± 0.04 P << .001
Control (21)	32.13 ± 0.17	10.63 ± 0.05

* Number of animals in each group.
† Mean standard error.

Godwin[7] using a radioactive isotope of astatine (At^{211}) which is the heaviest member of the halogen group. These investigators showed that PTU treatment resulted in a very marked enhancement of At^{211} accumulation by the thyroid which was in contrast to the decreased accumulation of I^{131} by the thyroids of PTU-treated rats.

TABLE 5. THE UPTAKE OF RADIOACTIVE THALLIUM (Tl^{204}) BY THE ADRENALS OF RATS TREATED WITH PROPYLTHIOURACIL

Hours after Tl^{204} injection	*Per cent dose x 10^{-3}*		*Per cent dose/mg. wet weight x 10^{-4}*		*Per cent dose/mg. dry weight x 10^{-3}*	
	Propylthiouracil	*Control*	*Propylthiouracil*	*Control*	*Propylthiouracil*	*Control*
½	9.5 ± 1.2* (3)†	10.5 ± 2.5 (2)	3.5 ± 0.5	3.6 ± 0.7	1.0 ± 0.1	1.0 ± 0.2
1	17.8 ± 2.8 (2)	14.9 ± 3.9 (3)	6.9 ± 0.9	4.5 ± 1.1	2.0 ± 0.2	1.4 ± 0.3
3	13.6 ± 2.7 (3)	15.8 ± 2.2 (3)	5.0 ± 0.9	4.9 ± 0.6	1.5 ± 0.2	1.5 ± 0.2
8	20.8 ± 0.9 P < .01 (2)	15.2 ± 0.8 (3)	7.9 ± 0.2 P < .01	4.2 ± 0.4	2.4 ± 0 P < .001	1.2 ± 0.1
24	12.6 ± 1.5 (2)	11.3 ± 0.5 (3)	4.8 ± 0.3 P < .01	4.0 ± 0.1	1.3 ± 0.1	1.2 ± 0.04
48	7.4 ± 0.3 (2)	7.0 ± 0.5 (3)	2.6 ± 0.2	2.3 ± 0.1	0.7 ± 0	0.6 ± 0.04
96	4.9 ± 0.3 (3)	3.5 ± 0.5 (3)	1.6 ± 0.1 P < .02	1.1 ± 0.1	0.5 ± 0.04	0.4 ± 0.04

* Mean ± standard error.
† Number of animals in each group.
"P" not recorded unless it is .05 or less.

The level of Tl^{204} in whole blood and plasma did not appear to be affected by PTU, approximately three times as much of the isotope being found in whole blood as in plasma (Table 2). Even though the Tl^{204} content of the thyroids did not appear to diminish appreciably during the 96-hour observation period, the blood and plasma levels of the isotope, when compared to the half-hour level, had fallen approximately 50 per cent by 24 hours and by 96 hours had decreased to about one-fifth of the half-hour values.

The uptake of Tl^{204} by the pituitary resembled that of the thyroid in that there appeared to be little diminution in isotope content during the course of the experiment (Table 3). In fact, at 96 hours the quantity of Tl^{204} in the pituitary was more than twice as high as at a half-hour. Although in some cases treatment with PTU appeared to increase the

TABLE 6. THE UPTAKE OF RADIOACTIVE THALLIUM (Tl^{204}) BY THE THYROID OF RATS TREATED WITH THYROTROPIC HORMONE FOR TWO DAYS PRIOR TO THE ADMINISTRATION OF Tl^{204}

Hours after Tl^{204} injection	*Per cent dose x 10^{-3}*		*Per cent dose/mg. wet weight x 10^{-4}*		*Per cent dose/mg. dry weight x 10^{-3}*	
	Thyrotropic hormone	*Control*	*Thyrotropic hormone*	*Control*	*Thyrotropic hormone*	*Control*
12	4.8 ± 0.4*	2.7 ± 0.06	3.5 ± 0.2	2.9 ± 0.3	1.7 ± 0.09	1.2 ± 0.09
	P < .01		P < .001		P < .01	
	(4)†	(4)				
24	3.7 ± 0.3	2.1 ± 0.3	2.7 ± 0.2	2.1 ± 0.09	1.3 ± 0.07	0.9 ± 0.04
	P < .01		P = .01		P = .001	
	(4)	(4)				
48	3.6 ± 0.5	2.0 ± 0.2	2.9 ± 0.4	2.2 ± 0.2	1.2 ± 0.1	0.8 ± 0.05
	P < .02				P < .05	
	(4)	(4)				
72	2.4 ± 0.2	1.6 ± 0.2	1.8 ± 0.03	1.8 ± 0.2	0.7 ± 0.01	0.7 ± 0.09
	P < .05					
	(4)	(3)				

* Mean ± standard error.
† Number of animals in each group.
"P" not recorded unless it is .05 or less.

pituitary uptake of Tl^{204}, the differences were probably not statistically significant.

Treatment with PTU resulted in a small but highly significant decrease in the wet and dry weights of the adrenals (Table 4) which confirms the observations of Leblond and Hoff[3] and Leathem.[4] However, PTU did not appear to affect the Tl^{204} uptake by the adrenals (Table 5). Although the Tl^{204} content of the thyroid and pituitary appeared to remain the same during the experiment, the Tl^{204} content of the adrenals did decrease at 96 hours to values 50 per cent or less than those at a half-hour.

Thyrotropic hormone. When rats were treated with large doses of TSH for two days prior to the injection of Tl^{204}, the uptake of the isotope by the

thyroid was significantly increased at 12, 24, and 48 hours (Table 6). By 72 hours, even though the thyroid content of Tl^{204} in the TSH-treated animals was still greater than in the controls, the differences were probably not statistically significant. In this connection it is interesting to note that Hamilton and Soley[3] were able to demonstrate an increased uptake of At^{211} by the thyroid of rats following treatment with TSH.

TABLE 7. THE UPTAKE OF RADIOACTIVE THALLIUM (Tl^{204}) BY THE THYROID OF RATS TREATED WITH THYROTROPIC HORMONE BEGINNING 12 HOURS AFTER THE ADMINISTRATION OF Tl^{204}

Hours after first injection of TSH	*Per cent dose x 10^{-3}*		*Per cent dose/mg. wet weight x 10^{-4}*		*Per cent dose/mg. dry weight x 10^{-3}*	
	Thyrotropic hormone	*Control*	*Thyrotropic hormone*	*Control*	*Thyrotropic hormone*	*Control*
3	4.4 ± 0.1* (4)†	4.1 ± 0.3 (4)	5.4 ± 0.3	4.7 ± 0.7	2.3 ± 0.1	2.0 ± 0.3
6	5.1 ± 0.1 (4)	3.8 ± 0.6 (4)	5.2 ± 1.0	5.1 ± 1.0	2.6 ± 0.6	2.0 ± 0.4
12	4.9 ± 0.3 P < .1 > .05 (4)	3.6 ± 0.6 (4)	5.9 ± 0.3 P < .02	4.3 ± 0.4	2.3 ± 0.1 P < .05	1.7 ± 0.2
24	3.7 ± 0.3 P < .05 (4)	2.6 ± 0.4 (4)	4.1 ± 0.2	3.8 ± 0.6	2.1 ± 0.2	1.7 ± 0.2
48	3.0 ± 0.4 (5)	2.1 ± 0.3 (5)	2.8 ± 0.4	2.9 ± 0.3	1.6 ± 0.2	1.3 ± 0.2
72	2.6 ± 0.2 P = .02 (5)	1.8 ± 0.2 (5)	2.7 ± 0.2	2.4 ± 0.3	1.4 ± 0.1 P = .02	1.0 ± 0.1

* Mean ± standard error.
† Number of animals in each group.
"P" not recorded unless it is .05 or less.

Data (not presented) for the Tl^{204} content of whole blood, plasma, pituitary, and adrenals after pretreatment with TSH showed no significant differences between the two groups of animals.

When rats began receiving injections of TSH 12 hours after the administration of Tl^{204}, the thyroid content of the isotope increased (Table 7). However, the magnitude of the increase was small and not significantly different consistently. The Tl^{204} content of whole blood, plasma, adrenals, and pituitary did not appear to be affected by TSH (data not presented).

SUMMARY

The metabolism of radioactive thallium (Tl^{204}) was studied in rats treated with either propylthiouracil (PTU) or thyrotropic hormone (TSH). PTU treatment resulted in an increased uptake of Tl^{204} by the thyroid, both on an absolute and on a weight basis. Although TSH did not release Tl^{204} from the thyroid, pretreatment with the hormone resulted in a significant increase in the Tl^{204} content of the gland.

The Tl^{204} content of whole blood, plasma, adrenals, and pituitary did not appear to be affected by PTU or TSH.

REFERENCES

1 Barclay, R. K., Peacock, W. C., and Karnofsky, D. A.: Distribution and excretion of radioactive thallium in the chick embryo, rat, and man. J. Pharm. Exp. Ther., 1953, *107*, 178.

2 Durbin, P. W., Hamilton, J. G., and Parrott, M. W.: Effect of pretreatment with propylthiouracil on accumulation of Astatine^{211} by thyroid gland of the rat. Proc. Soc. Exp. Biol., N. Y., 1954, *86*, 369.

3 Hamilton, J. G. and Soley, M. H.: A comparison of the metabolism of iodine and of element 85 (eka-iodine). Proc. Nat. Acad. Sc. U.S., 1940, *26*, 483.

4 Leathem, J. H.: Influence of thiourea on plasma proteins and organ weights in the rat. Endocrinology, 1945, *36*, 98.

5 Leblond, C. P. and Hoff, H. E.: Effect of sulfonamides and thiourea derivatives on heart rate and organ morphology. Endocrinology, 1944, *35*, 229.

6 Sharpe, L. M., Culbreth, G. G., and Klein, J. R.: The blood and packed cell volume of the adult rat as measured by tagged cells. Proc. Soc. Exp. Biol., N. Y., 1950, *74*, 681.

7 Shellabarger, C. J. and Godwin, J. T.: Studies on the thyroidal uptake of astatine in the rat. J. Clin. Endocr., 1954, *14*, 1149.

8 Thyresson, N.: Experimental investigation on thallium poisoning in the rat. Acta derm. vener., Stockh., 1951, *31*, 3.

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The Board of Editors

EVENTS

BOTANY-ZOOLOGY JOURNAL CLUB

February 16, 1955

HISTOCHEMICAL STUDIES ON DIFFERENTIATION IN THE CELLULAR SLIME MOLDS. By John T. Bonner, Department of Biology, Princeton University.

The life history of *Dictyostelium discoideum* can be depicted as follows: the germination of a spore gives rise to an amoeba which may continue reproducing by binary fission for two days. Thereafter, from 1000 to 100,000 individuals aggregate to form a sausage-shaped organism almost 1 mm. in length which may migrate for as many as twenty days. The stimulus for aggregation appears to be a chemical product of the cells, acresin. Migration is succeeded by the production of a vertical stalk and the formation of a fruiting body. It has been shown that the spore cases are formed by the posterior group of cells of the "sausage," while those anterior grow through the center of the organism to form the vertical stalk.

No differences between the anterior and posterior cells are apparent until the migratory stage. At this time, hematoxylin reveals dark granules in the presumptive spore cells, which the others lack. Nile blue sulfate becomes differentially distributed, too, and alkaline phosphatase activity is higher anteriorly, with the maximum occurring in the region of presumptive stalk cells at a time when stalk formation has begun. These presumptive cells are aligned on either side of the growing stalk and are presumed to produce its cellulose covering. Upon depletion of their polysaccharides, these cells become part of the incipient stalk.

Division of the sausages into anterior and posterior halves, all transitional material being removed, leads to the differentiation of two normal individuals. Thus, differentiation of anterior and posterior cells appears not to be irreversible at this stage.

Other species of slime molds are radically different. *D. mucoroides* is without a migrating stage, while *P. violaceum* forms a branched stalk, each branch bearing spores. In the former, presumptive stalk and spore cells are represented by a gradient rather than as two distinct zones, while in the latter there appears to be no distinct morphological difference between the two cells at all. It may be that this species represents a more elaborate form, due to the cells remaining embryonic or undifferentiated until the last minute in development. Thus, species-differences may be ascribed to differences in the timing of developmental events.

P. K.

CARDIOVASCULAR STUDY UNIT

March 7, 1955

SOME ASPECTS OF THE PULMONARY CIRCULATION IN MAN. By Alfred P. Fishman, Chest Service, Columbia University Division, Bellevue Hospital, New York, New York.

The primary concerns in the work reported are the capacity of the pulmonary circulation to regulate itself, and the mechanisms by which this

is accomplished. The observations were made on comfortably resting, fully conscious human patients. The techniques included arterial punctures, cardiac catheterization, and spirometry.

Previous animal work has shown increases in pulmonary artery pressure (with steady left auricular pressure) due to both increased CO_2 and decreased O2 partial pressures in the blood. The present work, including 27 subjects, indicates that in normal subjects, a low CO_2 blood tension leads to an increase in systemic pressure, with no change in pulmonary artery pressure or cardiac output. In a number of subjects with chronic emphysema there was greater variation in the results, some showing increases in both pulmonary artery pressure and cardiac output. Subjects with chronic silico-tuberculosis showed marked increases in pulmonary artery pressure and cardiac output. The conclusions can be drawn that there are no increases in pulmonary artery pressure without concomitant increases in cardiac output, and that with a restricted vascular bed (silico-tuberculosis) there will be increased pulmonary pressure and cardiac output.

Experiments designed to test the accuracy of the estimation of cardiac output show that calculations made by the direct Fick method five minutes following the beginning of low O2 breathing are inaccurate in that the organism is not in a steady state, as indicated by the fact that the RQ is still rising at that time. By the end of 17 minutes both the RQ and the cardiac output have stabilized, and the cardiac output is shown to be increased on low O2 breathing, whereas the measurements at the end of five minutes in the unstable state had shown a decreased cardiac output.

Eight subjects with decreased O2 blood tension consistently showed increased pulmonary artery pressures and increased cardiac output. Therefore the question arises as to whether the increase in cardiac output can account for the increase in pulmonary pressure. In experiments designed to test this hypothesis, it was found that in the normal individual an increase in cardiac output does not significantly increase the pulmonary artery pressure. In patients with pneumonectomy or chronic silico-tuberculosis, the increased cardiac output does lead to significant increase in pulmonary pressure. In addition it was shown that in cases where exercise increased both cardiac output and pulmonary artery pressure, hypoxia also increased the cardiac output and with a relatively greater increase in pulmonary artery pressure. Therefore, it can be concluded that the cardiac output is not the only factor involved in increasing the pulmonary artery pressure.

Evidence from pre- and post-sympathectomy did not indicate that the sympathetic nervous system was involved. Experimentation did not show that either epinephrine or histamine was involved in increasing the pulmonary artery pressure. Another possibility is that local hypoxia might be a factor causing a local vasoconstriction in the hypoxic lung. An experiment was then devised whereby it was determined that with one lung getting O2 to saturation and the other lung getting low O2 (by differential bronchospirometry), there was no shift in the volume of blood carried by either lung, therefore no change in the vascular resistance in the hypoxic lung. Decreased O2 in both lungs, however, gave definite increases in both cardiac output and pulmonary artery pressure. Therefore, it can be con-

cluded that in its mechanism of action, hypoxia leads to decreased arterial saturation, thereby leading to increased cardiac output, and thus to increased pulmonary pressure.

The conclusion from all the evidence is that the cardiac output contributes to the increase in pulmonary pressure but cannot account for all of it. Neither neurogenic nor local vascular responses (to hypoxia) appear to be involved in the experiments reported.

Other factors that may possibly be involved in the genesis of the increased pulmonary artery pressures in anoxia include: (1) volume elasticity characteristics of the pulmonary arterial tree, (2) static and dynamic blood volume changes, (3) ventilatory variations, (4) effects of extravascular and intrapulmonary structures on volume elasticity characteristics of pulmonary vessels, (5) vasomotor control, (6) communications between systemic and pulmonary circulation.

P. B.

YALE MEDICAL SOCIETY

March 14, 1955

RENAL FAILURE AFTER SHOCK. By Mark A. Hayes, Department of Surgery.

The use of comparatively simple renal function tests may give evidence of functional kidney impairment following periods of shock, even though oliguria or other clinical signs of acute renal failure are absent. Twelve of a consecutive series of 21 post-shock patients showed no impairment of either glomerular filtration, as approximated by urea clearance, or tubular function, as measured by the Fishberg concentration test. The remaining nine who did show impairment had exhibited systolic pressures lower than two-thirds of their post-operative recovery pressures for over one hour during the course of the shock period. Indeed, the time required for return to normal appears proportional to the length of time in shock, and roughly proportional to the severity of renal damage, as measured by the immediate post-shock urea clearance. Both tests show approximately the same range of 24-295 days before normal values return. Use of these simple tests may prove significant in evaluation of operative risk in patients who are scheduled for several consecutive operative procedures.

R. I. B.

THE EFFECT OF ADRENAL CORTICAL HORMONES ON THE CARBOHYDRATE METABOLISM OF THE LIVER. By C. N. H. Long, W. W. Winternitz and R. Dintzis, Department of Physiology.

In spite of their widespread use in clinical medicine very little is known of the nature of the action of the adrenal cortical hormones, except that the therapeutic effects are associated with their ability to increase the liver glycogen content and accelerate the catabolism of tissue proteins. In regard to this gluconeogenetic effect it is an unsettled question whether this is a consequence of an accelerated tissue protein catabolism which results in the

presentation to the liver of an increased quantity of glycogen precursors, or whether it represents a primary effect of these hormones on some phase of carbohydrate metabolism in the liver itself.

The present studies have been of two kinds. In the first the capacity of the liver of fasted normal, adrenalectomized, and adrenalectomized rats pretreated with hydrocortisone to form liver glycogen from several substances known to be glycogen precursors has been tested. This was done by the intravenous infusion over a 1½ hour period of standard quantities (80-100 mgs. per 100 g. of rat an hour) of glucose, fructose, glycerol, lactate, and malate. In all cases the proportion of substrate converted into liver glycogen was much less in adrenalectomized as compared to normal rats, being reduced to 42 per cent of normal for glucose, 46 per cent for fructose, 20 per cent for glycerol, 6 per cent for lactate, and zero per cent for malate. Pretreatment with hydrocortisone restored glycogen formation to normal although it did not do so entirely in the case of glycerol and lactate.

Since there was no evidence of any failure to convert these substrates to phosphorylated intermediaries such as glucose 6-phosphate, nor of any difficulty in removing them from the body fluids, it is concluded that in adrenalectomized rats a larger proportion of these substrates is utilized over some other pathway than that resulting in liver glycogen formation.

In the second series of experiments the liver of normal and adrenalectomized rats was presented with the necessity of utilizing an increased quantity of lactate consequent upon the subcutaneous injection of epinephrine. This agent increases glycogenolysis both in liver and muscles, the product in the latter being lactate, which is normally converted by the liver to glycogen and glucose (the Cori cycle).

There are striking differences in the response of normal and adrenalectomized rats to epinephrine. The fasted adrenalectomized rat, although it develops an equal degree of hyperglycemia, fails to deposit any significant quantity of liver glycogen. Furthermore, since the loss of muscle glycogen is nearly twice as great, there exists an unaccounted-for deficit in the carbohydrate balance which is some 100 mgs. per 100 g. of fat greater than is found in normal rats either one, four, or eight hours after epinephrine injection. This is not due to any failure to remove lactate from the blood and represents the diversion over some at present unknown metabolic pathway of a very considerable proportion of the muscle glycogen mobilized by the action of epinephrine.

Treatment of adrenalectomized animals with adrenal cortical extract brings about a normal response to epinephrine in which muscle glycogen loss is nearly balanced (four hours after injection) by the increase in liver glycogen and the accumulation of glucose in the body fluids.

This difference in response is not due to the increased insulin sensitivity of the adrenalectomized animals since adrenalectomized-diabetic rats show the same unaccounted-for loss of muscle glycogen when compared with diabetic rats. Nor is it due to an increased sensitivity of the muscle glycogen of adrenalectomized animals since the loss of muscle glycogen after epi-

nephrine injection is the same in eviscerated adrenal demedullated animals as it is in eviscerated and totally adrenalectomized rats.

It is concluded that adrenal cortical steroids of the type of cortisone and hydrocortisone have a primary effect on some phase of carbohydrate metabolism in the liver of a kind so that in their absence a large proportion of carbohydrate precursors which normally accumulate as glycogen and blood glucose are metabolized over some other pathway. This is equivalent to saying that access to these alternate pathways is regulated by these hormones. At the moment there is no definite evidence what these pathways may be nor do the present experiments exclude the possibility that these adrenal steroids have additional effects on tissues other than the liver.

M. M. L.

PHI DELTA EPSILON ANNUAL LECTURE

March 21, 1955

MANAGEMENT OF CONGESTIVE FAILURES. By Harry Gold, Professor of Clinical Pharmacology, Cornell University Medical College, New York, New York.

During congestive failure a factor of renal origin gives rise to a metabolic disarrangement of salt and water balance which assumes an autonomous character and remains independent of cardiac directed therapy.

In the past, congestive failure was thought solely to be a state of myocardial weakness. Recently, emphasis has been placed upon the phenomenon of fluid retention. Although restoration of cardiac efficiency may be the ultimate goal, proper treatment requires that we think in terms of a hyperhydrated patient.

A study which was conducted at the New York Hospital over a ten-year period and involving 500 patients employed the principles of effective digitalization and vigorous diuresis to the point at which the patient reached the so-called "dry" weight stage. In order to maintain proper fluid and calorie intake, patients on admission were placed on diets of milk and water, given alternately at hourly intervals and totaling 2500 to 3000 cc. per day. Concurrently digitalization was instituted along with a flexible regimen of organic mercurial diuretics.

It was observed that in 90 per cent of the cases receiving this initial treatment weight decline began within a short period of time, as excess fluids were excreted. Improvement in symptomatology paralleled weight loss. When treatment was continued, the body weight was found to reach a stationary level. At this point the diet was liberalized and a low salt intake (3-4 gms. per day) was maintained. Diuretic agents were continued, but the intervals between administration were increased. Although the treatment involved large initial doses of organic mercurials, no renal irritation was encountered, nor did any electrolyte disturbances arise which would force a modification of the scheme of treatment. This also applied to patients in uremia. The sole determinant for instituting the technique of therapy was the existence of congestive heart failure.

Although results obtained from this investigation are encouraging, it must be emphasized that the long-term success of this plan of treatment depends upon the strict co-operation of the patient.

R. H. F.

PLANT SCIENCE SEMINAR

March 22, 1955

GENETIC STUDIES USING CHLAMYDOMONAS REINHARDI. By William Ebersold, Dartmouth College.

Genetic data from the single celled algae have been accumulating since the controversial work of Moewus on *Chlamydomonas eugametos* was published over twenty years ago. Because of the fact that Moewus' cultures were not available, work on *Chlamydomonas reinhardi*, an alga with a life cycle of twelve to fourteen days, was begun. This alga is heterothallic, showing a one-to-one segregation for sex ratio, and in addition has the ability to grow in the dark on acetate-enriched medium.

Mutations were induced in plated cells by ultra-violet radiation, and the cultures were grown on minimal medium in the dark to prevent photoreactivation. They were then screened out by checking individual colonies or by using Lederberg's replica-plate technique. In addition to morphological mutants and streptomycin resistance, ten mutants involving growth factors were observed. These included requirements for nicotinic acid (whose actions followed the biochemical scheme of Neurospora), arginine, thiamine, para-amino benzoic acid, folic acid, and acetate. All of these mutants showed one-to-one segregation when crossed to the wild type and could be placed in two linkage groups.

Two ultra-violet-induced mutations were used to investigate Moewus' claim for crossing-over in the two-strand stage of meiosis. Experiments involving an arginine-requiring strain and a pale green strain showed crossing-over to be limited to the four-strand stage at both 5° and 25° C. Moewus also reported that a high salt concentration favored crossing-over at the two-strand stage in *Botrydium*. Results from experiments with *C. reinhardi* in nine-hundredths per cent sodium chloride may be interpreted as indicating either crossing-over at the two-strand stage or as chromatid interference. No definite statement can as yet be made.

RALPH HILLMAN

ZOOLOGICAL JOURNAL CLUB

March 23, 1955

STUDIES ON CERTAIN DIGESTIVE PROPERTIES IN INSECTS. By Thomas Eisner, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

In the course of evolution four main ways of increasing extracellular digestive capacity have originated. The first three, increase in length, in

diameter, and in folding of the digestive tract, all serve to enlarge the total surface area. The fourth way involves a different principle. A large meal is obtained and stored, and the subsequent rate at which this food is allowed to pass through a small-surfaced digestive tube is controlled. In insects, the gut is divided into three regions: the foregut, the midgut, and the hindgut. The first and last arise embryonically as anterior and posterior ectodermal invaginations, whereas the midgut is formed from endoderm and serves as the principal area of digestion and absorption. The hindgut serves mainly as a water absorber. A large part of the foregut, the crop, is a storage organ.

Does the crop in the cockroach *Periplaneta* function only in storage or is it also capable of food digestion and absorption? It has been thought for a long time that perhaps some carbohydrate digestion and absorption is possible as a result of salivary enzymes carried into the crop with the ingested food, but the report by Petrunkevitch about fifty years ago that the crop epithelial cells contained osmophilic granules was not considered evidence for fat digestion in this organ. It was thought that the crop produced no lipase, and the possibility that lipase secreted in the midgut could pass anteriorly, thereby producing lipase activity, was precluded by the assumption that the proventriculus, at the junction of the foregut and midgut, served as an effective one-way valve. The presence of osmophilic granules in the crop epithelium, it was said, was due to fat distribution following digestion and absorption in the midgut. Sanford, however, by ligating the posterior end of the crop, showed that fat granules appeared only in the epithelial cells anterior to the ligature. Therefore, the crop is capable of absorbing fat.

Beautiful histological preparations indicated that the assumed one-way valve function of the proventriculus had no morphological basis. Thus three possibilities arose: either the crop secreted its own lipase, or the enzyme migrated anteriorly to the crop through the proventriculus, or fat was absorbed directly without digestion. Utilization of Gomori's histochemical procedure showed that lipase activity was present in the foregut, but that lipase secretion was limited to the midgut. Experimental evidence showed that lipase activity is maximum at a pH of approximately 8 and very low at pH5. Surprisingly, the crop has the latter pH value, so fat which is absorbed in this organ is nearly all undigested. Experimentally, the roach is most accommodating, for after each moult the crop epithelial cells are free from fat granules. Experiments carried out just after moulting showed that the crop epithelial cells incorporated oleic acid after two hours, light mineral oil after six hours, and heavy mineral oil only after five days. The two latter findings showed that there is a direct relationship between the rate of incorporation and the degree of oil viscosity. However, when oleic acid was mixed with heavy mineral oil, the time of absorption was once again short: only two to four hours. The presence of fatty acid in the crop thus greatly increases the rate of fat absorption. Even though the crop does not secrete lipase, the presence of the enzyme in this organ facilitates absorption of viscous fats by producing small amounts of fatty acid.

Finally, no fat granules were observed in the epithelial cells of the hind gut.

LINCOLN P. BROWER

BOOK REVIEWS

METHODS IN MEDICAL RESEARCH. Vol. 6, J. M. Steele, Editor-in-Chief. Chicago, The Year Book Publishers, Inc., 1954. xiii, 271 pp. $7.00.

This volume consists of four sections: the largest is on "Statistics in Medical Research" and is a general account of the non-technical aspects of the subject; a section on "Methods in Environmental Medical Research" makes up the second major portion of the book; and the volume is completed by two smaller sections, of some 30 to 40 pages each, on "Some Methods of Studying Human Genetics" and the "Design and Construction of Metabolism Cages." The variety of subject-matter is greater than in previous volumes of the series, though there is much material that will have a general appeal to workers in medical research.

Donald Mainland, with the help of his assistant, Lee Herrera, has prepared the chapters on statistical methods, and the work is characterized by the clear writing for which Mainland is known in this field. The purpose of the section is not to provide details about how to perform particular statistical calculations, but rather to give a "realization of what modern biological statistics implies throughout the conduct of any type of medical investigation." It is debatable, however, whether a worker in medical research is likely to learn much about statistics by studying general principles alone. For most people, the consideration of specific problems, especially when combined with the working of numerical examples, is the only way to develop an understanding of the wider issues, and, in fact, the authors have recognized this by using illustrations, including in some cases numerical data drawn from medical research of current interest.

Following the introductory chapter there is an account of what is meant by the effect of chance, or random, factors. An understanding of this is necessary in the analysis of results of experiments, but it is equally important in the design of experiments, for in this latter process one first excludes, or decides to make independent observations on, important systematic factors, and then, by the act of deliberate randomization, ensures that the remaining factors will affect the results in a random manner. It is, therefore, clear that the question of what is to be treated as a chance factor is controlled in part by the investigator, and it is equally clear that the design of the experiment must be married to the analysis of the results.

The two following chapters give a general account of the planning of investigations and the analysis in relation to the planning. The description is excellent and the examples apt, but there is an implied overestimate of the contribution which a statistician, as such, can make to the planning of an experiment. The basic issues in planning are still the subject-matter issues which center around the choice of a fruitful, explicit hypothesis, and the selection of the best experimental approach to the problem. These are prestatistical considerations, for the most part, and different degrees of experience, knowledge, and ability among investigators will be reflected in the suitability of the plan that is finally adopted. In short, there is much more to the designing of experiments than that portion of the subject which is discussed by statisticians, however helpful the contribution of these workers may sometimes be.

On page 145 there is an interesting comment on the danger of making comparisons for which the experiment was not designed. It is often not

realized that a hypothesis which is formulated after the data have been collected and inspected has a different status from one formulated before, and tested by, the experiment. The special uncertainty which adheres to the former type of hypothesis arises from the failure to take into account the vagaries of chance. An atypical outcome may have occurred, as a result of random factors, but the hypothesis formed on inspection of this outcome will seem just as convincing as an hypothesis founded on the typical outcome. When the hypotheses are formulated, as they should be, before the experiment begins, the researcher has good protection against accepting as real a relationship that has resulted merely from chance factors. As E. Bright Wilson has put it, the research worker, like the young player of billiards, should call his shots.

A chapter is devoted to the modern type of clinical trial in which the experimental method is rigorously applied to tests of therapeutic agents on human patients; as for example in the investigation, by the Medical Research Council of Great Britain, of the value of streptomycin in the treatment of tuberculosis. The problems which arise include some that are statistical in nature. Mainland has taken a special interest in this work and writes about it in an able and clear manner. In the following chapter a contrast is drawn between such clinical trials and the type of clinical survey which is based on a retrospective examination of hospital records. The numerous difficulties encountered in this latter method are examined. In the course of the discussion reference is made to "Berkson's Fallacy," which may arise not only in these surveys of hospital records but also in any investigation where a group of hospital patients with some disease is contrasted with a control group of patients in respect to some factor, such as quantity of tobacco smoked, or possession of a second disease. The essence of Berkson's argument is that the groups examined in hospital may not give an unbiased representation of the corresponding groups in the population. The hospital groups will be biased if there is a selective factor affecting the admission of patients with the disease, as compared with those without it, and of patients with the character under study, as compared with those without it. Thus two selective factors must operate; if one were sufficient, all such investigations on hospital patients would be invalid, because the presence of the disease clearly increases the probability that a person will enter hospital. The validity of Berkson's argument cannot be questioned; but some further investigation is needed to see how important it is in practice. For instance, an association between smoking and cancer of the lung has been shown both in the earlier retrospective studies on hospital patients and also in the later studies using follow-up methods on a section of the general population. Similarly, an association between maternal rubella and congenital defects in the child has been shown by both methods. Some effort should be directed towards defining more carefully any safeguards that can provide some protection against drawing invalid conclusions from retrospective studies.

A chapter on some undesirable effects of laboratory tradition is devoted to a discussion of five fallacious uses of figures. These incorrect usages are attributed, perhaps somewhat unjustly, to the influence of training in the

physical sciences. They arise in part from a failure to understand, and come to terms with, the variability which characterizes biological measurements.

An important chapter is provided on the problem of the independence of observations. It is a common mistake to regard each of, say, three measurements made on subject A as being equivalent to a single measurement made on B. Again, in chemical work, so-called duplicate measurements are often made without complete independence, so that the difference between them indicates only a portion of the error of the method. It is a reflection on the high standards usually maintained by these workers that the term "chemist's duplicates" is taken to imply that the two observations are dependent to some extent, and therefore not true duplicates at all.

There are chapters on "Confidence Limits," "Standard Deviations and Standard Errors," "Sample Sizes" and "Standards of Significance," and, in these, common statistical procedures and ideas are explained and illustrated. There is also a chapter on non-parametric tests; that is to say, tests in which, for example, ranks might be used instead of measurements. If measurements have actually been made, this results in some loss of information, but the counterbalancing gain is simplicity and avoidance of complicated assumptions such as that of normality.

This section should be supplemented by a book which describes the techniques commonly used in statistical work and which provides more numerical examples; but it is a stimulating contribution to the subject. The writing is somewhat didactic at times, and there are occasional excursions into matters of philosophy and organization which the reader may be invited to take or to leave.

The remaining three sections of the book are not examined critically in this review but the contents are given for the sake of completeness.

The section on "Environmental Medical Research" includes chapters on the "Measurement of Climatic Variables," "Respiratory Exchange," "Energy Metabolism and Metabolic Reference Standards," Radiometric Methods for Measurement of Skin Temperature," and "Measurement of Sweating." In selecting these topics a special attempt was made to concentrate on those parts of the general field that had not been previously discussed in the series.

The section on "Human Genetics" is limited to four topics which are each described by Dr. C. C. Li: "Segregation of Recessive Offspring," "The Severity of an Abnormality," "Methods for establishing the Genetic Role," and "Linkage Versus Association."

In the final section on "Metabolism Cages" there is a very extensive account of various cages used for work on rats, mice, dogs, and monkeys. There are detailed instructions for building the cages and descriptions of some special techniques for which they are used.

COLIN WHITE

RECENT PROGRESS IN HORMONE RESEARCH. Vol. X. Gregory Pincus, Ed. New York, Academic Press Inc., 1954. 510 pp. $9.80.

The rapid advances in our knowledge of the endocrine system have made it increasingly difficult for even a student of a single organ to remain con-

versant with the work of his colleagues. The large annual meetings where several hundred papers may be "given" within a few days serve only as a general outline of work in progress, since the opportunity for critical discussion as well as correlation with the work of others has been sacrificed to the principle that all speakers have something equally important to say, so long as they say it in ten minutes.

This has led to the organization of a new type of meeting, exemplified by the conferences sponsored by the Macy Foundation and those of the Laurentian Hormone Conference. In these meetings a few chosen individuals whose work gives them authority to speak on a selected topic open, as it were, the discussion. This is followed by a period of questions and answers both between the speaker and his invited audience and the members of the audience themselves. Such a procedure not only ensures that the audience will have a judicial survey of the topic but also affords an opportunity for open criticism, addition, or expansion of the material presented. While some might argue that spontaneous discussion, or the hasty question and answer, should best remain unprinted, the fact is that careful recording and editing enable a much larger audience to feel that they had actually participated in the conference. Since such conferences must of necessity remain small if they are to accomplish their purpose, it is only fair that their deliberations should ultimately become available to all who wish to read them.

This is not an easy editorial task, as anyone knows who has attempted to translate such discussions from a recording tape. Nevertheless, this volume, like its predecessors, has retained to a remarkable degree the spontaneity which is such a refreshing aspect of these conferences. While many definitive answers to important problems will not be found in this volume, nevertheless the reader cannot fail to grasp both the direction of present efforts for their solution as well as the lines of investigation that are being pursued.

The present volume contains the initial papers and discussion of six topics considered at the 1953 conference. They are: I. Nervous system—hormone interrelationships, II. Thyroid hormone physiology and biochemistry, III. Comparative endocrinology, IV. Protein hormones, V. The role of hormones in blood and blood-forming organs, VI. Aspects of clinical endocrinology.

The relation of the nervous system to the hormones was discussed by R. W. Porter, H. Hoagland, and D. M. Woodbury. The recent studies on tri-iodothyronine are adequately summarized by J. Gross and Pitt-Rivers, while the *in vitro* effects of these hormones are well reviewed by H. Landy and G. F. Maley.

The present reviewer enjoyed the two papers on comparative endocrinology by D. Bodenstein and Dr. and Mrs. Scharrer. The preparation and chemistry of crystalline insulin was discussed by R. G. Romans, and the intricate chemistry of the several corticotrophines by E. E. Hays and W. F. White.

The rôle of various hormones in the control of experimental lymphomas was the subject of the paper by H. S. Kaplan, C. S. Nagareda, and M. B. Brown. Finally, Dr. B. Zondek pointed out some of the unsolved problems of endocrine function in pregnancy, while the effects of adrenal steroids and ACTH on the inorganic and organic metabolism in man were reviewed

in two papers, one by R. Luft and the other by J. W. Conn and their respective colleagues.

It is impossible in a short review to do justice to all these excellent papers. Suffice it to say that those who wish to learn of the most recent aspects of research in these fields of endocrinology cannot afford to ignore this volume. Since this is a yearly publication, many of the statements cannot reflect more than the opinions of the participants at the time they were given, and indeed many may well be altered by now. Nevertheless, in this day and age, with the present volume of papers rising to increasing levels, it is pleasant to know that by the use of this and similar volumes an investigator need not be more than a year behind the current thought in his field.

C. N. H. LONG

PSYCHOMOTOR ASPECTS OF MENTAL DISEASE. AN EXPERIMENTAL STUDY. By H. E. King. Cambridge, Harvard University Press, 1954. xiv, 185 pp. $3.50.

This is a clear presentation by the author of a series of well-planned studies of psychomotor performance. The experiments were suggested by the hope that tests of psychomotor function might distinguish between the different categories of psychiatric patients and even be a sensitive guide to the degree of psychological disability.

Tests were limited to those which measured fine movements. These included speed of initiating movement, tapping rapidity, manual and finger dexterity, and a more complex motor response termed the "disjunctive lift reaction." All were measured mechanically with considerable precision. These tasks were selected from a much larger group of procedures after it had been established by factor analysis that each of the four tests assessed relatively independent and unrelated skills in the motor area. Subjects who were studied included groups of normal, neurotic, pseudoneurotic, and chronic schizophrenic individuals. The numbers were large and the groups well matched.

The major finding was a very significant difference between the normal and schizophrenic subjects with a gradient between these extremes—the more disturbed the patients, the poorer in general were their performances. Chronic schizophrenics as a group reacted much more slowly and were less adept than all other subjects.

The differences were unequivocal, but the meaning of these discrepancies was not quite so clear. After discussing the possibility that chronic schizophrenics might have done poorly on these tests because of their emotional isolation, lack of motivation, inability to concentrate etc., the author discarded these explanations for reasons which were well argued but inconclusive. Instead he favored the view that the psychomotor impairment of the schizophrenics related to a basic maladaptation. Exactly what was meant was not clear to the reviewer, although it may be that Dr. King is referring to biological or psychological factors at the core of the schizophrenic process as yet unidentified.

No matter what the ultimate explanation of the data may prove to be, the study stands as clean-cut, thoughtful, thorough, and necessary. The presentation is orderly, the observations important, the discussion of the literature is excellent, and the statistics are pleasantly unpretentious yet adequate.

EUGENE BLISS

ISOTOPIC TRACERS. By G. E. Francis, W. Mulligan, and A. Wormall. New York, John de Graff, Inc., 1954. xvi, 306 pp. $7.00.

This volume might well serve as model for any laboratory course in the use of isotopes as tracers. The first section provides a clear and very concise survey of the principles upon which such use is based. The inclusion of many examples of practical technique may have resulted in some overconciseness of theoretical exposition, but the bibliography offers ample supplementation. The second section offers a series of well-thought-out and very practicable laboratory exercises illustrating a variety of applications of the technique to biochemical problems. These exercises, combined with the excellent section on hazards and precautions, and with the list of rules suggested for a biochemical tracer laboratory, offer to the neophyte the basis for a very long step toward competence in the field.

Unfortunately, the usefulness of this book as a text in this country is much reduced by its detailed restriction to apparatus currently available in the authors' own laboratories. One questions the desirability of any detailed descriptions of apparatus in a field showing such rapid advances in instrumentation. And in the present case this appears to have resulted in a serious slighting of a number of techniques which have already proved themselves in other laboratories. Counting in the proportional region and analysis by neutron activation are two striking examples of omission, while the discussion of scintillation counting certainly errs on the side of pessimism.

One can, however, unreservedly recommend the book for the consideration of anyone involved in teaching isotope methodology to biological or medical students.

TOTAL CARE OF THE SURGICAL PATIENT. The Surgical Clinics of North America. Symposium from the Pacific Coast Surgical Association. Philadelphia, W. B. Saunders Co., 1954. x, 1199-1491 pp.

The title of the *Total Care of the Surgical Patient* contained within such a limited monograph of necessity implies that specific aspects of total care are emphasized. These special aspects are handled in a most creditable fashion and the volume warrants very careful study by every physician caring for surgical patients. The section devoted to the use of adrenocorticoid hormones or their endogenous production by corticotrophin is a succinct presentation orienting their use towards definite indications; a similar practical discussion of the use of plasma, plasma expanders, blood transfusion, and blood fractions is presented.

The portions of the monograph devoted to fluids, electrolytes, and nutritional problems are presented in rather a sketchy fashion and in them-

selves constitute a somewhat inadequate and elementary review. Anesthesia, though generally considered a specialty of its own, is an adjuvant to operative treatments, and the skillful review presented here should be of interest to all surgeons; a companion presentation is the very pertinent presentation concerning surgical patients with cardiac, diabetic, or renal handicaps.

The discussion of thrombosis and embolism is considered to be one of the most worthwhile portions of the monograph. The author has taken a very fundamental and questioning attitude toward current concepts and provides the thoughtful reader with much to consider in basically altered physiology and biochemistry.

The highly specialized topics of problems related to certain areas of operative treatment are handled in manners varying from excellence to mediocrity. The significant ones appear to be those concerned with cardiac arrest, prevention and treatment of surgical infections, steroids in the management of advanced carcinoma, postoperative pulmonary complications, biliary tract operations, acute intestinal obstruction, lesions of the colon and rectum, urinary tract complications, and patients with multiple injuries.

In summary, the monograph is generally an excellent one for general review reading and should be available as references for all physicians with a surgical interest.

MARK A. HAYES

ANALYTIC STUDIES IN PLANT RESPIRATION. By F. F. Blackman. New York, Cambridge University Press, 1954. x, 231 pp. $7.50.

This volume is composed of a series of nine papers by the late F. F. Blackman. The first three papers are reprinted from the Proceedings of the Royal Society and are, therefore, more or less familiar to interested persons. The last six papers are taken from documents left in varying stages of completion by their author. With the consent of Mrs. Blackman, a former student of Blackman's, Dr. J. Barker, prepared the last six papers for publication.

The entire volume is devoted to the review and analysis of data obtained from a study of the respiration of 21 apples in the years 1920 and 1921. These apples, taken from uniform storage one by one, were subjected to various experimental conditions and the effects on respiration noted and recorded.

Many today would disagree with Blackman's "single apple" approach as being inadequate for a proper statistical analysis of the resultant data. The results, however, raise several questions which would have been obscured by the average values of the many apples used in a statistical approach to such a problem. The ripening apples gave respiration curves, as determined by carbon dioxide evolution, of three distinct types. The types were closely correlated with the ripening time of the apples concerned, early ripening apples giving one type of curve, late ripening apples another. This data indicated a considerable degree of physiological variation within the apple variety tested.

The same apples were used to determine respiration in nitrogen and in oxygen concentrations other than atmospheric. Again the same pattern of

physiological variation between the early, middle, and late ripening apples was apparent.

The deductions and reasoning from the experimental data by Blackman in an attempt to clarify the results make the book worthwhile even if one takes issue with his lack of a statistical basis for his conclusions. The work should, as a result, be entitled to a place in the classical researches into the complexities of plant respiration of its day.

A. R. COOPER

THE MICROTOMIST'S FORMULARY AND GUIDE. By Peter Gray. New York, The Blakiston Co. Inc., 1954. xiii, 794 pp. $10.50.

Seldom has a book come into my laboratory which was so readily utilized. The technician in my laboratory glanced at its contents and asked to borrow it. She returned it only after reading it completely with the comment that many little tips on routine work, so often taken for granted by teachers of, or books on, histological technique were clearly presented and were now being put to use during her daily tasks.

A colleague of mine had difficulty finding a lucid and complete description of how to prepare frozen sections in the popular technique books. Upon reading the typical example of the preparation of a frozen section in Gray's book he informed me that it contained the exact information he needed, presented in a logical, step-wise fashion from the beginning of the process to its completion without omitting little steps so often taken for granted in similar books. I share the favorable opinions mentioned above.

This work consists of two parts. Part I is a treatise on the art of making microscope slides from biological specimens. Part II is a classified list of the formulas and techniques used in this art. Part I could easily serve as a text in a histological technique course. It should also prove valuable to medical students at the Yale School of Medicine whose thesis work requires the preparation of histological material. The illustrations in this section are clear and adequate.

Part II contains virtually all solutions employed during the last 50 years. Each chapter is subdivided decimally and each individual method or formula is identified by its decimal classification with the originator's name and date. All duplication is thereby avoided and many thousand cross references are given without recourse to the index or numbered page. Pet names of preparations which are embedded in the folklore of microtechnique are put in italics after the proper name. Formulas have been adjusted to give a rational total (usually 100) in terms of standard ingredients, no matter how the original was presented. This wearisome labor applied to thousands of formulas by the author can well be appreciated by the reader.

The author is well qualified and his first edition contains surprisingly few errors in general makeup such as typographical errors, incorrect references to figures, etc.

E. S. CRELIN

RENAL FUNCTION. Stanley E. Bradley, Ed. Fifth Conference on Renal Function of the Josiah Macy, Jr. Foundation, October 1953. New York, The Josiah Macy, Jr. Foundation, 1954. 218 pp. $3.75.

This volume resembles the reports of previous conferences of the Josiah Macy, Jr. Foundation in combining detailed experimental data with free discussion by expert participants. In contrast to previous volumes this report is concerned primarily with experimental aspects of clinical problems. Three major topics were considered:

1. The discussion of the *nephrotic syndrome* is primarily a presentation of the excellent work of John A. Luetscher, Jr. The demonstration, identification, and purification of the salt-retaining corticoid of urine of nephrotic patients during edema and therapy were discussed. This fraction is probably aldosterone and is found in increased amounts in the urine during the salt-retaining phase of the disease (urine sodium concentration less than 10 mEq/L.).

2. *The problem of kidney transplantation* is a discussion of the natural history of homologous transplants in the thighs of humans with advanced renal disease. After an initial period of oliguria due to acute tubular necrosis, urine output from the transplanted kidney increased to large volumes. Clearance techniques demonstrated remarkably high glomerular filtration rates in the transplanted kidney for as long as several months.

3. Acute renal failure is a discussion by Graham MacGregor Bull of acute tubular necrosis and circulatory renal insufficiency. Pathogenesis and various aspects of treatment of anuria are considered.

This book, as are the other volumes on renal function, is invaluable to students of kidney physiology and disease.

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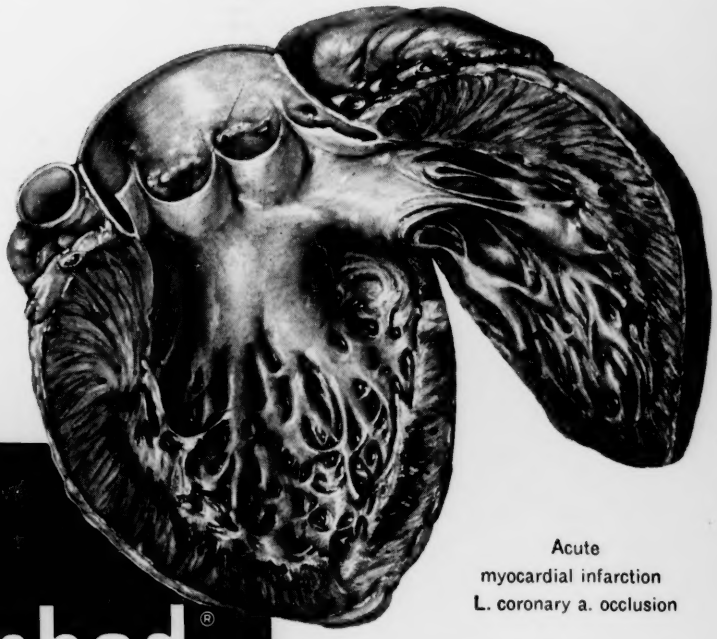

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1. Gazes, P. C., Goldberg, L. I., and Darby, T. D.: *Circulation*, 8: 883, Dec., 1953.

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